The effect of Dichloroacetate on oxygen uptake during sub-maximal and maximal exercise in humans

by

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For my Grandparents

Don't Quit

When things go wrong as they sometime will
When the road you're trudging seems all uphill,
When funds are low and debts are high
You want to smile but have to sigh,
When care is pressing you down a bit
Rest if you must but don't you quit.
Life is queer with its twists and turns
As every one of us sometimes learns,
And many a failure turns about
When he might have won had he stuck it out.
Don't give up though the pace seems slow
You may succeed with another blow,
Often the goal is nearer than
It seems to a faltering man.
Often the struggler has given up
When he might have captured the winner's cup
And he learned too late when the night slipped down
How close he was to the golden crown.
Success is failure inside out.
The silver lining of the clouds of doubt
You can never tell how close you are
It may be near when it seems so far,
So stick to the fight when you're hardest hit
It's when things seem worse that you mustn't quit.

Unknown Author

Abstract

Objective

Pyruvate dehydrogenase (PDH) is a multienzyme complex situated on the inner mitochondrial membrane. Its principal function is to catalyze the irreversible oxidation of pyruvate to acetyl CoA. PDH is activated by a compound known as dichloroacetate (DCA). The principal metabolic effect of DCA administration is an increased availability of acetyl-CoA and acetylcarnitine for substrate phosphorylation, and a lowering of blood [lactate]. Studies using DCA during exercise have identified the availability of these acetyl groups as a major determinant of $\dot{V}O_2$ increase at the start of exercise, suggesting a functional stenosis to $\dot{V}O_2$ may reside at the level of PDH. It was the aim of these studies to further investigate whether DCA has an effect on the rate and magnitude of the $\dot{V}O_2$ response for sub-maximal and maximal work intensities.

Procedures

A ramped incremental exercise protocol and 2 sub-maximal constant exercise protocols were undertaken on a cycle ergometer. The constant work protocols were undertaken at work intensities above and below that corresponding to the individual blood lactate threshold. Measures of breath-by-breath $\dot{V}O_2$ and heart rate were made in conjunction with measures of muscle oxygen saturation (NIRS) and blood borne indices (Lactate, pH, Glucose, Bicarbonate). $\dot{V}O_2$ kinetics were estimated using non-linear least squares regression strategies.

Results

Dichloroacetate had no significant effect on submaximal oxygen uptake. This was evidenced as no change in the amplitude or time course of the kinetic response during exercise above or below the lactate threshold, and no change in the slow component response above the lactate threshold. DCA administration did promote a small but significant increase in $\dot{V}O_{2max}$. Blood [Lactate] was reduced at submaximal exercise intensities. However, no significant difference was evident during maximal exercise. DCA was shown to have chronotropic properties evident by an observed increase in heart rate at rest and during the initial stages of exercise.

Conclusions

The present studies clearly illustrate a haemodynamic response to DCA that has not been described previously. Control of oxygen uptake kinetics at the level of pyruvate dehydrogenase is not supported by this work.

Acknowledgements

What I will remember the most from this journey are the people I've met along the way.

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Liam. You're a good mate. Cheers

Viki. Thank you for the tea! Good times always remembered.

To my family. You are a foundation and an inspiration. I always feel very lucky to have you.

Lastly to my wife Lisa, your faith in me kept me going when I had lost faith in myself. Finishing this says more about us than it does about me. I love you x

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Definitions of Abbreviations

Variables:

$\dot{V}O_2$	Oxygen uptake	l/min or ml/min
$\dot{Q}musO_2$	Muscle oxygen consumption	l/min or ml/min
O_2	Oxygen	
ATP	Adenosine tri-phosphate	mM
ADP	Adenosine di-phosphate	mM
Pi	Inorganic phosphate	mM
Eqn	Equation	
$\dot{V}O_{2\,max}$	Maximum oxygen uptake	l/min or ml/min
WR_{max}	Maximum work rate	W
WR	Work rate	W
$\dot{Q}p$	Cardiac output	l/min or ml/minϕ1
PCr	Phosphocreatine	mM
Cr	Creatine	mM
RER	Respiratory exchange ratio	
O_2df	The oxygen deficit	l/min or ml/min
$\dot{V}CO_2$	Carbon dioxide output	l/min or ml/min
$\dot{V}O_{2(SC)}$	Slow component of $\dot{V}O_2$	l/min or ml/min
DCA	Dichloroacetate	
PDC	Pyruvate dehydrogenase complex	
PDH	Pyruvate dehydrogenase	
TCA	Tricarboxylic acid cycle	
NIRS	Near Infrared Spectroscopy	
MVC	Maximum voluntary contraction	
$C(a-\bar{v})O_2$	Arterial-venous oxygen difference	ml/min
$\dot{V}E/\dot{V}O_2$	Ventilatory equivalent for oxygen	l/min or ml/min
$\dot{V}O_{2LT}$	$\dot{V}O_2$ at lactate threshold	

Parameters:

τ	(Tau); 'fundamental' time-constant	s
τ'	(Tau prime); 'mean-response time'	s
δ	delay time (parameter in kinetic models)	s
$\phi I, \phi II, \phi III$	Phase I, II and III of the $\dot{V}O_2$ response	s
θ_L	Estimated Lactate Threshold	l/min of $\dot{V}O_2$
CP	Critical Power	W
W'	Curvature constant of the power-duration relationship	
$\dot{V}O_{2\ 6\text{-}3}$	Minute 6- minute 3 of the $\dot{V}O_{2(SC)}$	

Definitions:

W	Work Rate	W
['X']	Concentration of 'X'	
Δ	Change (i.e. $\Delta\dot{V}O_{2(t)}$ = change in $\dot{V}O_2$ over time t)	
P	Partial pressure (i.e. PO_2 = partial pressure of oxygen)	mmHg
F	Fractional concentration (i.e. FO_2 = Fractional oxygen content)	%
C	Concentration (i.e. $C\bar{v}O_2$ = venous oxygen content)	
'X'$_{ss}$	Steady state of 'X'	
'X'$_{xs}$	'Excess' of 'X' (i.e. $\dot{V}O_{2(XS)}$ excess oxygen uptake)	

Chapter 1

Introduction

Chapter 1

1.0 Introduction

Since the end of the 19th century, measurement of inspired and expired gases and calculation of the gas exchange for oxygen has been used as a measurement of pulmonary oxygen uptake. With advances in technology, these calculations can now be reliably performed over a single breath.

Breath-by-breath measurement in response to dynamic work rate forcing functions has led to the characterisation of consistent temporal patterns of response in pulmonary oxygen uptake. Furthermore, the fundamental $\dot{V}O_2$ kinetic response has shown to be a close approximation to that of muscle oxygen consumption ($\dot{Q}musO_2$), and it presents an opportunity to infer readily and reliably the metabolic adaptation to changes in energetic demand (Barstow *et al.*, 1990; Grassi *et al.*, 1996; Whipp *et al.*, 1982).

Muscle oxygen uptake increases in response to an augmented energetic requirement, to sustain muscle contraction and cell homeostasis. Appropriate modelling and interpretation of the pulmonary $\dot{V}O_2$ response profile to a work rate forcing can express the characteristics of this underlying system response and ensure the inferences made are not unfounded.

Armed with an understanding of the "normal" or "typical" $\dot{V}O_2$ response to exercise, it is possible to investigate modulators of this response. Inferences can then be made as to

the putative mechanism or mechanisms that may be controlling the adaptation to a change in energy demand.

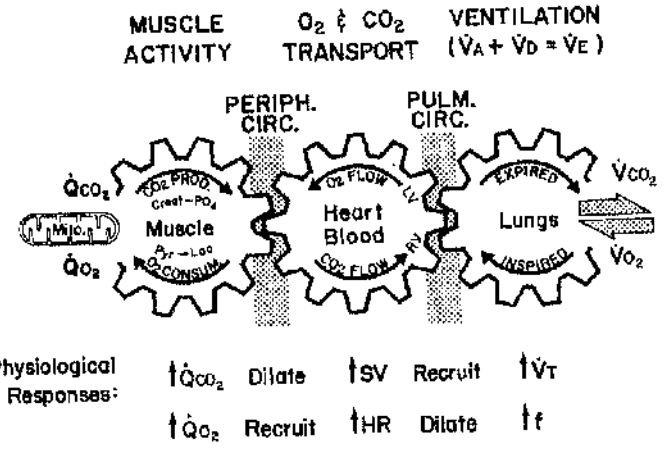


Figure. 1 - Diagram illustrating the gas transport system for coupling cellular to pulmonary respiration. The gears represent functionally independent, interlocked components of the system. An increase in O_2 utilisation by the muscles is met by an increased extraction of O_2 from each of the links in the oxygen transport system (from Wasserman *et al*, 1987).

The respiratory system, the cardiovascular system and the exercising muscle cells are functionally interlocked and interacting systems that transport oxygen from the lungs to the muscle to support aerobic ATP resynthesis. This allows oxidative phosphorylation and muscular work to continue (fig.1). Disruption of any component of these systems can lead to an alteration in the gas exchange response and affect the efficiency and relative intensity of the muscular work performed. For example the muscular work performed as a large part of everyday life can in the diseased state, where the capacity to perform exercise can be severely limited, prove to be a major barrier to improving quality of life and long term health prospects. Conversely, in an athletic population, an improved performance may lie in understanding how the key components govern the exercise response and how these components respond to training.

Therefore further understanding of the mechanism(s) governing the $\dot{V}O_2$ response to exercise may have significant impact in health and disease.

1.2 Exercise Intensity Domains

The importance of appropriately describing the intensity of exercise when discussing oxygen uptake kinetics will become clear in the following chapter. Four recognised but not undisputed intensity domains, Moderate, Heavy, Very Heavy and Severe (Whipp, 1996) are the preferred method of description for the study of $\dot{V}O_2$ kinetics. The gas exchange and metabolic (blood lactate and pH changes) responses to constant 'square wave' exercise are distinct for each. Therefore these measures provide a basis for defining the intensity of the exercise.

The range of work rates that do not result in a sustained increase in arterial blood lactate concentration and subsequently decrease arterial pH is termed the 'moderate' domain i.e. the work is being undertaken below the lactate threshold. In this region, $\dot{V}O_2$ will increase to a steady state value in approximately 3 min, in response to a step change in exercise workload. An individual, in good health, exercising in this domain should feel no great discomfort in his/her breathing or any great tiredness in his/her legs (fig. 2).

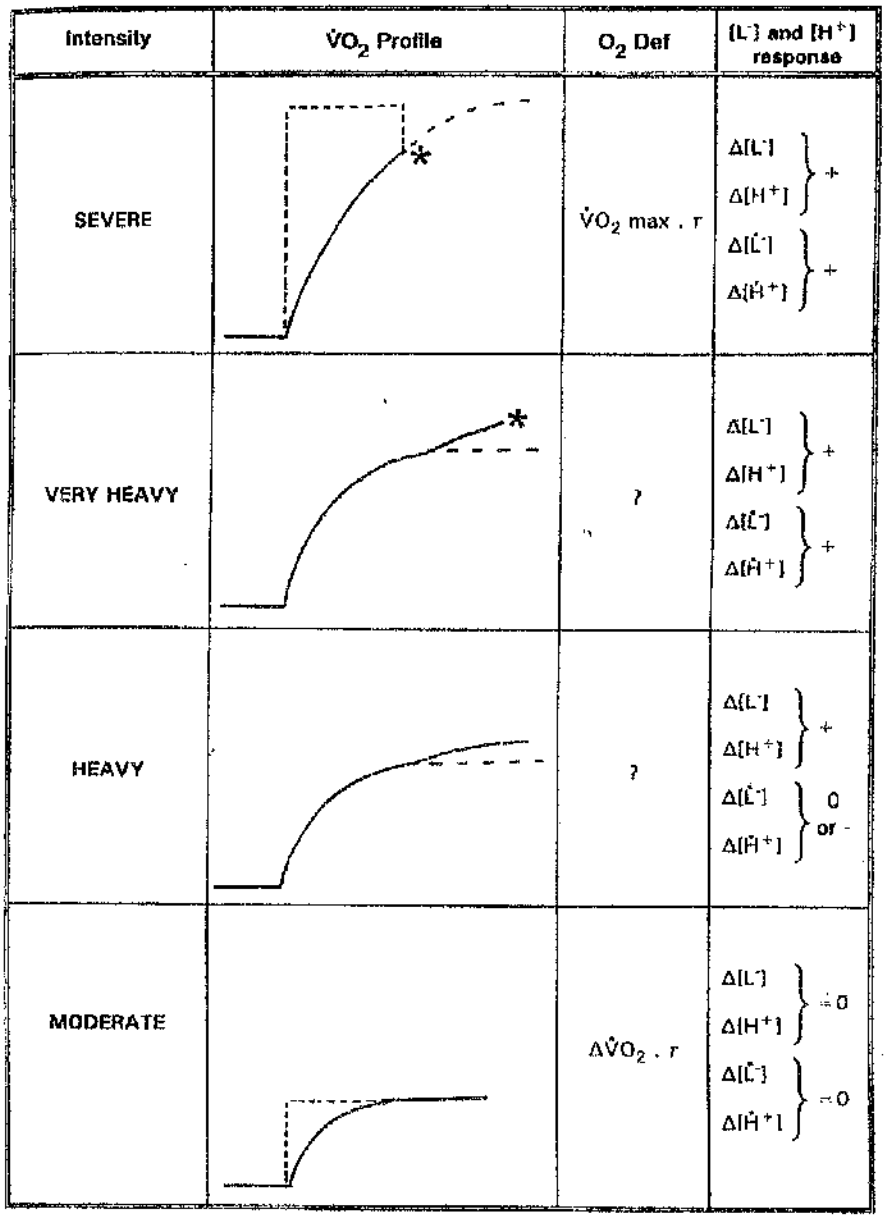


Figure 2 - A schematic representation of the temporal response of $\dot{V}O_2$ to constant-load 'square wave' exercise at different work intensities. Δ [L⁻] = change in blood lactate. Δ [$\dot{L}^-$] = change of rate of change in blood lactate. Δ [H⁺] = change in hydrogen ion concentration. Δ [$\dot{H}^-$] = change of rate of change in hydrogen ion concentration. Moderate: below θ_L; there are no sustained increases in [L⁻] or [H⁺]. $\dot{V}O_2$ reaches a steady state. Heavy: above θ_L; $\dot{V}O_2$ reaches a steady-state but with a delayed time course. Increases in [L⁻] and [H⁺] are sustained. Very Heavy: above θ_L; $\dot{V}O_2$ eventually reaches a maximum due to the contribution of the 'excess $\dot{V}O_2$'. Severe: supra-maximal (i.e. WR above an incremental WR_{max}) work rate where fatigue occurs so rapidly that the 'excess $\dot{V}O_2$' has not had time to develop discernibly. * indicates $\dot{V}O_2$ max. The term O_2Def is described in section 1.5 (from Whipp, 1996).

During 'heavy' intensity exercise there is a sustained increase in arterial blood lactate and a sustained decrease in arterial pH. The responses in both stabilise or even decline. Exercise in this domain has therefore exceeded the lactate threshold. $\dot{V}O_2$ in this domain can reach a steady state, the attainment of this steady state is however delayed by as much as 10-15 minutes (Poole *et al.*, 1991, Ozyener *et al.*, 2001).

Arterial blood lactate and arterial pH changes do not stabilise in the 'very heavy' exercise domain. They both increase throughout the duration of the exercise to a maximum. The end point to a continuous exercise protocol in this domain is fatigue, with high levels of dyspnoea and tiredness in the legs evident. $\dot{V}O_2$ follows a similar response to lactate in that it fails to stabilise i.e. a steady state is not reached. From the start of exercise $\dot{V}O_2$ continues to rise throughout until the maximum ($\dot{V}O_{2max}$) is reached (fig. 2).

The 'severe' domain is the range of work rates above the maximum achieved by an individual during an incremental exercise protocol to exhaustion (WR_{max}). These are termed supra-maximal work rates as the maximum associated with an incremental test can be superseded during shorter duration 'square wave' or intermittent work. Peak arterial lactate values and decreased arterial pH are associated with severe leg fatigue and very heavy breathing in the severe domain. $\dot{V}O_2$ increases rapidly to $\dot{V}O_{2max}$ and predictably does not reach a steady state. Fatigue ensues and work rates can only be maintained for short periods (fig. 2).

The advantage of assigning exercise intensity in this way is that each domain reflects distinct physiological responses. These responses are functionally similar across

individuals, in that they are bound by parameters that adapt to proportional changes in aerobic and anaerobic responses to stimuli such as training or disease.

Assigning exercise intensity in this way is more appropriate than using the more commonly used percentage of maximum as each domain describes better the expected physiological demands and responses. For example a work rate assigned as a percentage of maximum oxygen uptake ($\%\dot{V}O_{2max}$) will be impossible to maintain as a constant $\%\dot{V}O_{2max}$ if the work rate lies within the very heavy or severe domain. The inability of the $\dot{V}O_2$ response to reach a steady state in these domains means that a percentage of $\dot{V}O_{2max}$ is not sustainable.

1.3 Critical Power

Critical power is functionally significant because it corresponds to the highest WR at which a $\dot{V}O_2$ steady state can be attained (Poole *et al*, 1990., Hill *et al*, 2002) and coincides with the highest WR at which arterial [L^-] and [H^+] do not continue to rise throughout the work (Poole *et al*., 1988) i.e. it represents the border between the 'heavy' and 'very heavy' exercise domains (fig. 3).

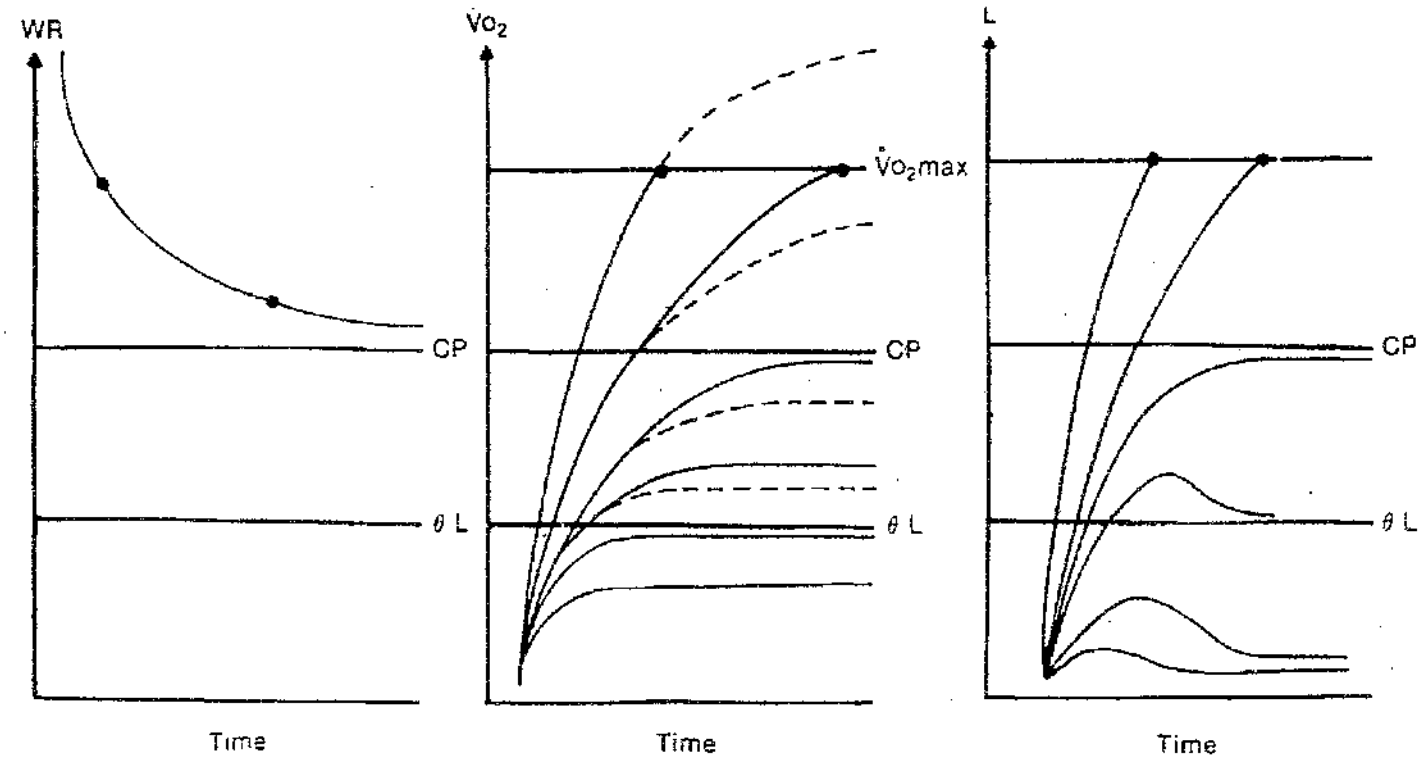


Figure. 3 - Left panel: Power-duration relationship for high intensity exercise (i.e., > critical power, CP. Refer to section 1.3). Centre panel: Basic features of $\dot{V}O_2$ kinetics for constant-load exercise. The solid lines represent observed $\dot{V}O_2$ responses. The dashed lines represent the $\dot{V}O_2$ response predicted from a linear $\dot{V}O_2$ work relationship. For work rates below the lactate threshold (θL), $\dot{V}O_2$ increases exponentially to a new steady state. For work rates between θL and CP, $\dot{V}O_2$ stabilises at a value higher than that predicted from the underlying exponential (dashed line). For higher work rates, $\dot{V}O_2$ does not stabilise but attains $\dot{V}O_{2max}$. Right panel: Equivalent profiles of blood [lactate] responses; note the sustained elevation for work rates between θL and CP, but the progressive increase for supra-CP work rates (taken from Whipp, 1994).

In their early work Hill and Lupton (1923) described a level of exercise where lactic acid and $\dot{V}O_2$ continued to rise throughout exercise until fatigue set in. Margaria *et al* (1965) stated that this rise to $\dot{V}O_{2max}$ is achieved progressively faster with increasing work rate, a finding later supported by Wasserman *et al* (1967) and Whipp (1994). It has been clearly shown that there is a range of work rates where $\dot{V}O_2$ cannot stabilise and continues to rise until $\dot{V}O_{2max}$ is reached and fatigue ensues, fig. 3 (Poole *et al.*, 1988). This demarcation point for $\dot{V}O_2$ from a sustainable steady-state has been termed critical power (Moritani *et al.*, 1981) for cycle ergometry or the 'fatigue threshold' (Hughson *et al.*, 1984) for treadmill running and 'critical velocity' for swimming (Wakayoshi *et al.*, 1992).

This relationship between power (P) and time to fatigue (t) has been well described by a number of authors as a hyperbolic function (Monod and Scherer, 1965; Moritani *et al.*, 1981; Hughson *et al.*, 1984; Poole *et al.*, 1988; Poole *et al.*, 1990; Miura *et al.*, 2002), similar to equation 1.

$$(P - Cp) \cdot t = W'$$

Eqn 1.

Where Cp (or $P_{l.t.}$ in fig. 4) is the power asymptote termed 'critical power' and W' is the curvature constant (fig. 4). This relationship is often transferred into its linear formulation (Whipp *et al.*, 1982; Poole *et al.*, 1988; Hill, 1993):

$$P = (W'/t) + Cp$$

Eqn 2.

where W' and Cp are the slope and intercept respectively (fig. 4).

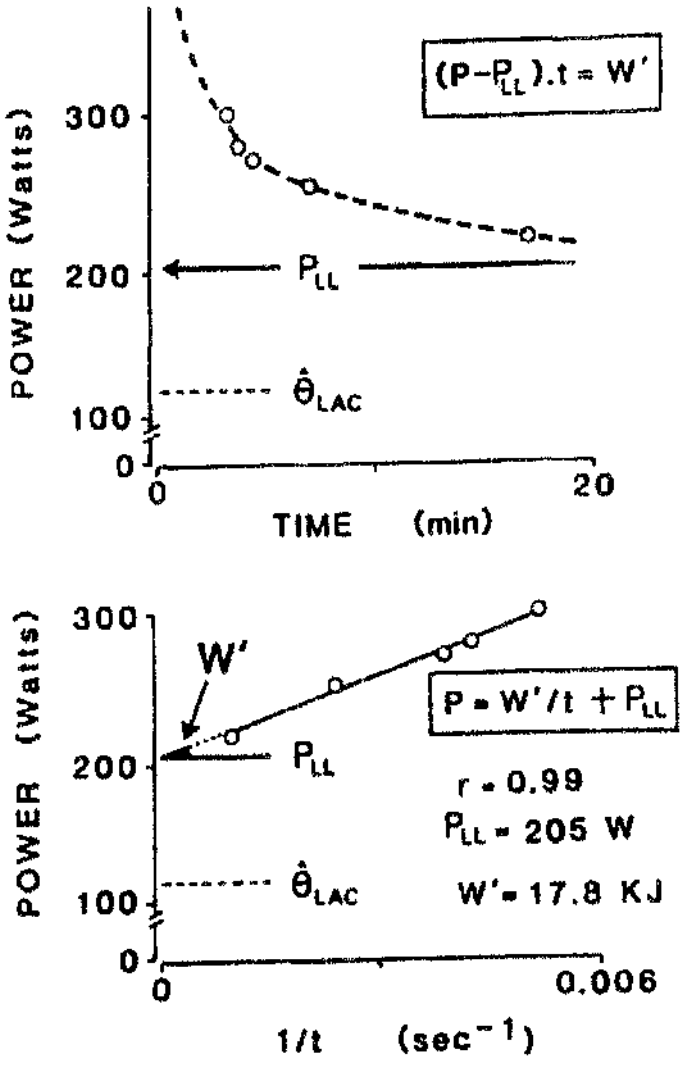


Figure 4 - Upper diagram: The power-duration relationship for high intensity exercise. Lower diagram: Determination of parameters W' and > P_{LL} from the linear P – 1/t formulation. θ_{la^-} is the estimated lactate threshold using the 'V-slope' (Ventilatory threshold) method described by Beaver *et al* (1986). (Poole *et al*., 1988).

Poole *et al* (1988) and Whipp and Ward (1990) have described the curvature constant parameter W' as an equivalent to a constant amount of work that can be performed above Cp regardless of the rate at which it is performed. Therefore in this region $\dot{V}O_2$ becomes a function of both time and power and the relationship between work rate and $\dot{V}O_2$ (refer to section 1.8.2.) is discontinued.

At work rates below critical power-'moderate' and 'heavy' domains- $\dot{V}O_2$ is able to sustain a steady state. Therefore Cp represents, in theoretical terms, the upper limit for continuous constant load exercise of almost indefinite duration (Moritani *et a.*, 1981) i.e. ATP resynthesis can be met by the transport and utilisation of oxygen. In practice this is not quite realised as the simple hyperbolic relationship between power and time breaks down for very long or very short exercise (for review see Hill, 1993).

Poole *et al* (1988) found Cp to lie on average at approximately 70% of maximal work rate (from a standard incremental work test) or 46% of the difference between the work rate corresponding to an estimated lactate threshold and work rate max. However Poole *et al* (1990) demonstrated that Cp is a trainable parameter, showing a 10% improvement in Cp after 7 weeks of endurance training. This finding was supported by Jenkins and Quigley (1992) who showed a 30 % increase in Cp after 8 weeks of endurance training.

Therefore Cp is an important 'threshold' when regarding exercise intensity, one that is not immovable and appears to be closely linked to factors that respond to endurance training i.e. blood [Lactate] and acidosis (Poole *et al.*, 1988).

1.4 Sub-threshold $\dot{V}O_2$ kinetics

When regarding the temporal response of $\dot{V}O_2$ to a change in exercise intensity it is beneficial to contemplate first the factors influencing oxygen uptake at the lung.

Fundamentally, pulmonary $\dot{V}O_2$ is well described by the rate at which partially reduced haemoglobin enters the pulmonary capillary bed. The affinity of haemoglobin to bind oxygen is governed by a number of factors (i.e. pH, temp., PO_2). Its delivery in to the lung is a function of the pulmonary blood flow $\dot{Q}p$ ($\dot{Q}p$ = Cardiac output in healthy individuals) and the mixed venous oxygen content ($C\bar{v}O_2$). These variables can be related by the Fick equation for measuring cardiac output, rearranged here in terms of pulmonary $\dot{V}O_2$:

$$\dot{V}O_2 = \dot{Q}p(CaO_2 - C\bar{v}O_2)$$

Eqn 3.

Here CaO_2 denotes arterial oxygen content.

With this relationship in mind let us consider three recognised 'phases' (Whipp *et al.*, 1982) of the temporal $\dot{V}O_2$ response to a step change in work rate within the moderate domain.

1.4.1 'Phase 1'

At the onset of constant-load exercise there is an initial rapid increase in $\dot{V}O_2$ that has been termed "Phase 1" (ϕ1) (Whipp *et al.*, 1982). This initial increase is thought to be dominated by changes in cardiac output since the effect of any change in muscle metabolism on $C\bar{v}O_2$, will not be expressed as a change in $\dot{V}O_2$ until the muscle venous effluent has passed to the lung vasculature. An increase in cardiac output at the onset of exercise, predominantly due to an increased stroke volume, results in an increase in pulmonary blood flow ($\dot{Q}p$) and hence an increase in $\dot{V}O_2$ *(Eqn.* 3). The nature of the increase in $\dot{V}O_2$ during this initial phase has led to it being described as cardiodynamically mediated (Whipp *et al.*, 1982).

This phase of the response was theoretically proposed by Krogh and Linahard in 1912, however direct measurement was only established in 1966 by Achincloss *et al* who directly recorded an increase in $\dot{V}O_2$ from the onset of exercise. The rapid increase in $\dot{V}O_2$ can account for up to 50% of the $\dot{V}O_2$ response in this domain and is essentially invariant with respect to the magnitude of the step in work rate (Weissman *et al.*, 1982). Support for the cardiodynamic nature of this early response has come from a demonstration of proportionality between the early $\dot{V}O_2$ and $\dot{Q}p$ responses (Cummin *et* al., 1986 and Miyamoto *et al.*, 1982). Furthermore, factors affecting pulmonary blood flow at the onset of exercise have been shown to modulate the rate and magnitude of the $\dot{V}O_2$ response. Attenuation of the size of the change in stroke volume at exercise onset by performing the exercise from a background of unloaded pedalling (Whipp *et al.*, 1982) or in a supine position (Weiber-Ravell *et al.*, 1983; Karlsson *et al*, 1975) have

both been shown to evoke a similar attenuation of ϕI $\dot{V}O_2$ response. Stietsema *et al* (1986) demonstrated a diminished ϕI $\dot{V}O_2$ response in patients with cyanotic congenital heart disease, a disease that results in little or no increase in pulmonary blood flow at the onset of exercise (fig. 5. Upper graphs). A similar finding has been found in patients with chronic obstructive pulmonary disease (COPD) (Nery *et al.*,1982) (fig. 5. Lower graphs).

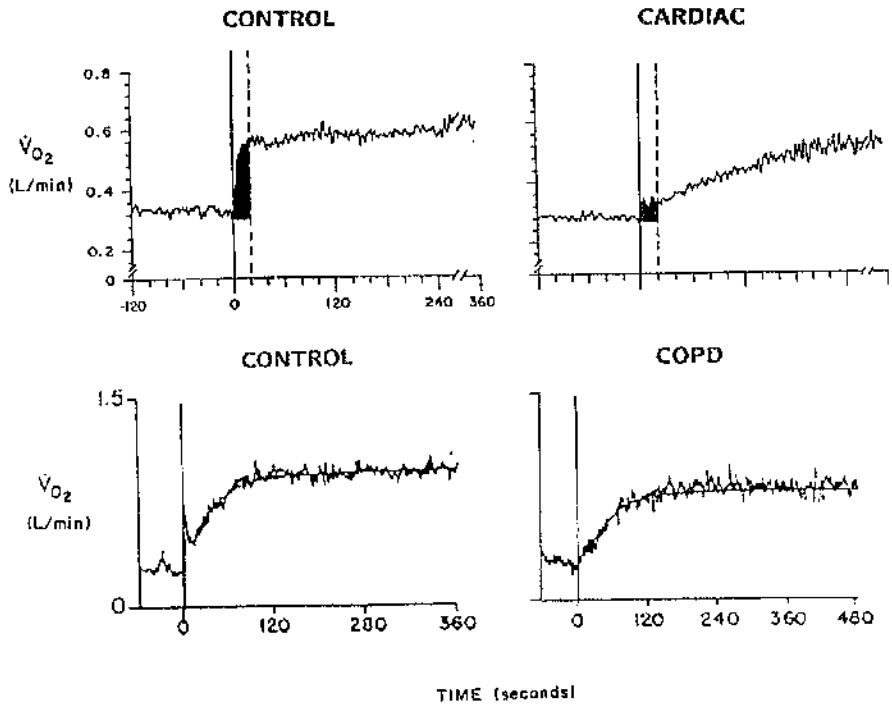


Figure 5 - Temporal response of oxygen uptake ($\dot{V}O_2$) response to moderate square-wave exercise performed from a background of unloaded pedalling for a patient with cardiac disease (top right; taken by Whipp and Ward, 1990; from Stietsema *et al.*, 1986) and a patient with chronic obstructive pulmonary disease (COPD) (bottom right; taken by Whipp and Ward, 1990; from Nery *et al.*, 1982).

1.4.2 'Phase II'

After the initial rapid increase during phase one, $\dot{V}O_2$ continues to rise in a mono-exponential fashion until it reaches a plateau (Henry, 1951; Davies, 1972; Di-Prampero, 1970; Linnarsson, 1974; Whipp, 1971; Wasserman and Whipp, 1974; Hughson and Morrissey, 1982; Ozeyner *et al.*, 2001). A widening of the arterial-venous oxygen difference is the dominant factor modulating pulmonary $\dot{V}O_2$ during this phase; i.e. CaO_2 remains constant at the onset of exercise while muscle venous and subsequently mixed venous oxygen content decrease. This additional component to gas exchange only exerts an influence once the vascular transit delay from muscle to lung has passed. The temporal domain within which these changes take place has been termed "phase 2" (oII) (Whipp *et al.*, 1982; fig. 6, panel C).

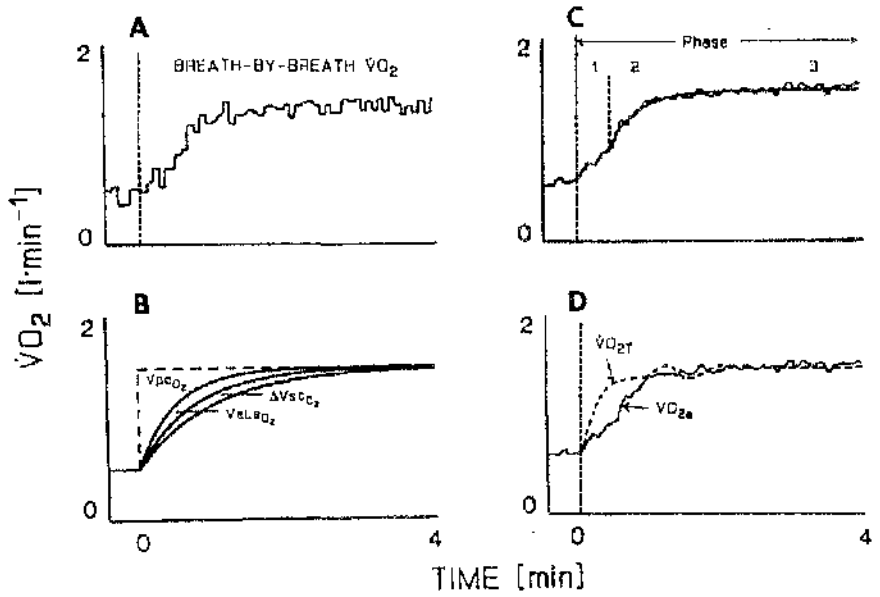


Figure 6 - (A) Pattern of oxygen uptake ($\dot{V}O_2$) response to a square wave change in work rate of moderate intensity. (B) Traditional view where $\dot{V}O_2$ rises mono-exponentially without delay; the venous O_2-stores (ΔV_{st} O_2) and a transient lactate O_2-equivalent (Ve_{La} O_2) is also accounted for. (C) $\dot{V}O_2$ response divided into three phases (refer to text for further explanation). (D) estimation of 'mean tissue O_2 consumption'. Note that B and D lead to obligatory conclusion that $\dot{V}O_2$ kinetics are slower than muscle oxygen uptake kinetics (Barstow *et al.*, 1990).

In their pioneering work Krogh and Lindhard (1912) proposed that there would be a delay of at least 6-10 s before venous blood leaving the muscle reached the lung and that any changes in ventilation or gas exchange prior to this would be as a result of changes in $\dot{Q}p$. This transit delay of mixed venous blood from the muscle to the lung has subsequently been demonstrated to be in the order of 10-15 s (Cerretelli *et al.*, 1966).

The muscle oxygen consumption ($\dot{Q}O_2$) response to moderate exercise in isolated muscle preparations has been shown to follow a mono-exponential rise from the onset of exercise with little, or no, delay (Mahler,1985). Similarly the phase II rise in pulmonary $\dot{V}O_2$ can be characterised by an exponential of the form:

$$\Delta\dot{V}O_{2(t)} = \Delta\dot{V}O_{2(ss)}(1-e^{-kt})$$

Eqn 4.

Where $\Delta\dot{V}O_{2(t)}$ represents the pulmonary oxygen uptake above baseline any time (t) after the onset of exercise, $\Delta\dot{V}O_{2(ss)}$ is the steady-increment, and k is the rate constant of the reaction (Whipp, 1971). The slope of the $\dot{V}O_2$ response is given by the rate constant k. The faster the response is, the greater the value of k.

When describing the φII $\dot{V}O_2$ response the rate of increase in $\dot{V}O_2$ is often used. Commonly however the parameter referred to is the time constant of the response (τ). The time constant is the reciprocal of the rate constant i.e. $1/k$. For a first-order system, one time constant represents the time taken to reach 63% of the steady state response. A long time constant represents a slow response, a short time constant represents a

faster response. Similarly the half time for the response is often quoted and the three parameters are related thus ($\tau = 1/k = t1/2 / 0.693$).

The exponential nature of the pulmonary $\dot{V}O_2$ response was described in the early work of Henry (1951) and Henry and De Moor (1956) (fig. 7). Margaria *et al* (1965) first described this time course as a monoexponential function with a half time of about 30s. Their work has subsequently found support for moderate intensity exercise (Cerretelli *et al.*, 1966; Davies *et al.*, 1972; Whipp, 1971; Whipp and Wasserman, 1972; Linnarsson, 1974; Hagberg, 1978; Whipp *et al.*, 1982; Ozeyner *et al.*, 2001). The time constant for the $\dot{V}O_2$ response ($\tau\dot{V}O_2$) in a young healthy adult is typically approximately 20-40 s (Whipp, 1971; Whipp and Wasserman, 1972; Davies *et al.*, 1972; Bakker *et al.*, 1980; Whipp and Ward, 1980; Whipp *et al.*, 1982; Hughson and Morrissey, 1982). Whipp and Mahler (1980) reported invariance in $\tau\dot{V}O_2$ for different work rate changes in the moderate domain. However it does tend to be shorter in trained subjects (Hagberg *et al.*, 1980), and longer in elderly sendentary subjects (Babcock *et al.*, 1994) and patients with cardiorespiratory disease (Puente-Maestu *et al.*, 2001).

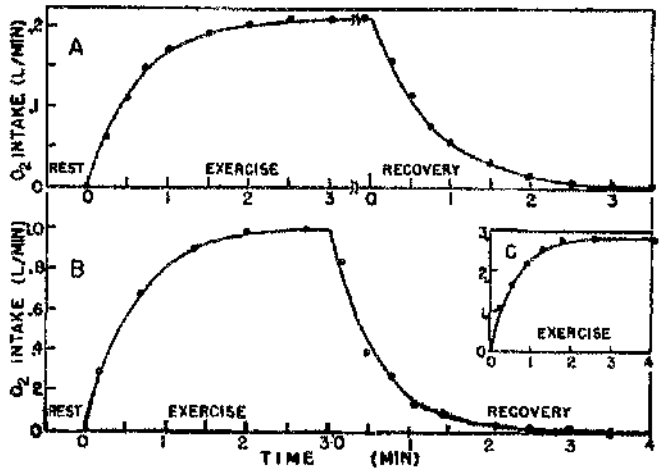


Figure 7 – Early work by Henry, (1950) showing the $\dot{V}O_2$ kinetics for both the on and off transient using 15 s Douglas bag sampling. The mono-exponetial characteristic of the responses is clearly shown.

The mono-exponentiality of the $\dot{Q}musO_2$ response as demonstrated by Mahler, M. (1985) and Meyer (1988) is similar to, yet not exactly, that that is expressed as $\dot{V}O_2$ during 'phase II'. This is due to the value of $C\bar{v}O_2$ established at the muscle being associated with a higher blood flow by the time it is expressed at the lung i.e. blood flow will have increased during the muscle to lung transit time. However, Barstow *et al* (1990) modelled the relationship between an increase in $\dot{Q}musO_2$ and the subsequent increase in pulmonary $\dot{V}O_2$. They found that the time constant for muscle oxygen uptake was likely to be less than 10% different from $\tau\dot{V}O_2$. Grassi *et al* (1996), by taking $\dot{V}O_2$ across the leg as a close approximation to $\dot{Q}musO_2$, found the rate of change for pulmonary $\dot{V}O_2$ and $\dot{Q}musO_2$ to be similar ($T_{1/2}$ 25.5 $\pm$ 2.6 s and 27.9 $\pm$ 5.7 s for alveolar and leg $\dot{V}O_2$ respectively). Therefore, while separated by a necessary phase in time, the time course and amplitude of the phase II pulmonary $\dot{V}O_2$ response is a close approximation to $\dot{Q}musO_2$. Inferences made on the temporal response of muscle oxygen consumption from measurements of $\dot{V}O_2$ are therefore not unfounded. To consider these inferences however a suitable modelling strategy must be adopted and a number of assumptions made (refer to section 1.7).

1.4.3 'Phase III'

After approximately 3 min, or essentially 4 time constants, $\dot{V}O_2$ reaches a plateau phase of the response (in a young healthy adult). There is no further significant increase or decrease in the mean $\dot{V}O_2$ and the response is said to have reached a 'steady state' (Phase 3, (ϕ3)). In the phase the change in steady state $\dot{V}O_2$ ($\Delta\dot{V}O_{2(ss)}$) is equal to the

mean rate of oxygen utilisation at the muscle ($\Delta\dot{Q}musO_{2(ss)}$) i.e. the energy requirement of ATP resynthesis in the exercising muscle is being met aerobically (Whipp *et al.*, 1982). The $\Delta\dot{V}O_{2(ss)} / \Delta WR$, or steady-state gain, for moderate cycle ergometry exercise is remarkably consistent at approximately $10ml \cdot \min \cdot W^{-1}$ across individuals regardless of age or training status (Wasserman and Whipp, 1975; Hansen *et al.*, 1984). It only appears to increase in the obese individual due to the increased work of moving the legs (Wasserman and Whipp, 1975).

1.5 The O_2 deficit and O_2 debt for moderate intensity exercise

During the steady state the transport and utilisation of oxygen meets the metabolic demands of the muscle. However it is clear that a step change in work rate and therefore a step change in demand for ATP resynthesis is not met by a step change in $\dot{V}O_2$. This 'short-fall' or 'lag' in the $\dot{V}O_2$ response is has been termed the oxygen deficit (Christensen and Hogberg, 1950). Figure 8 illustrates this concept. The area bound by the $\dot{V}O_2$ on-transient response and the solid lines, represents the portion of the energy requirement not met by O_2 delivery and utilization.

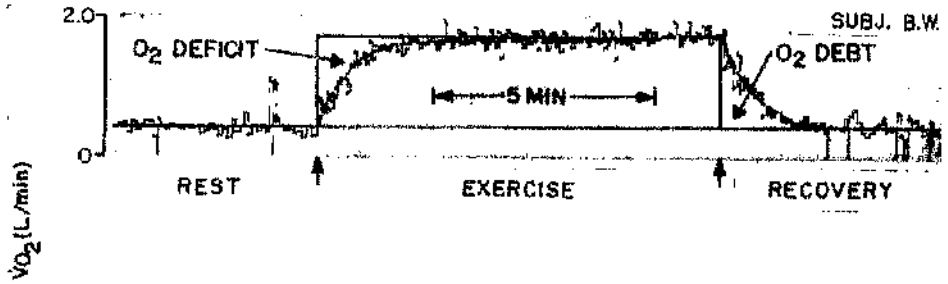


Figure 8 - Temporal $\dot{V}O_2$ response for a normal subject during a 125 W square wave exercise forcing (exercise undertaken at a work intensity below the lactate threshold). The solid line indicates the work rate change. Areas of O_2 deficit and O_2 debt are labelled (Whipp and Wasserman, 1972).

This shortfall in aerobic energy yield is met or 'buffered' by the breakdown of phosphocreatine (PCr), the utilisation of oxygen stored in the muscle bound to myoglobin and oxygen contained in the vascular system (Whipp *et al.*, 1982; Rossiter *et al.*,1999). The predominant energy buffer is PCr hydrolysis. The temporal response profile of PCr breakdown is essentially dynamically symmetrical to that of $\dot{V}O_2$ and has led to suggestions for a linked mechanism of control of $\dot{V}O_2$ through the PCr $\leftrightarrow$ Cr (creatine) reaction (refer to section 1.11.7, fig. 17) (Meyer, 1988; Rossiter *et al.*,1999).

The oxygen deficit is functionally significant as it represents the systems ability to 'go into debt' (Hill and Lupton, 1923) in order to undertake exercise that even in this moderate domain would not be possible if the lag in $\dot{V}O_2$ was not compensated for. The size of the oxygen deficit is a product of the effective time constant (τ', the result of a mean response exponential fit through phase I and II, see section 1.7) and the gain in steady-state $\dot{V}O_2$ ($\Delta\dot{V}O_{2(ss)}$) (Equation 5. Whipp and Ward, 1993).

$$O_2def = \tau' \cdot \Delta\dot{V}O_{2(ss)}$$

Eqn 5.

Therefore circumstances that lengthen τ, such as chronic heart failure, COPD, inactivity and old age (Babcock *et al,* 1994), increase the size of the O_2 deficit. Therefore there is a greater reliance in these patients on finite energy sources. Conversely these energy stores are to an extent spared in the more athletic population where the time constant of the response has been reported to be reduced (Hagberg *et al.*, 1978, Hagberg *et al.*, 1980).

At the cessation of exercise the $\dot{V}O_2$ above that of the new steady state requirement has been termed the 'oxygen debt' as it represents the additional oxygen cost of replenishing the energy 'borrowed' during the on-transient of the response. Figure 8 illustrates this region as the area beneath the off-transient response. In the moderate domain where off- and on-transient responses are symmetrical (Patterson and Whipp, 1991; Ozyner *et al.*, 2001), the size of the O_2 deficit and O_2 debt are approximately equal (Whipp *et al.*, 1970; Whipp and Wasserman, 1972).

1.6 System Linearity

It has been proposed that the phase II $\dot{V}O_2$ kinetic response to moderate exercise is first order and dynamically linear (Whipp and Wasserman, 1972). That is, in adjusting to a change in work rate, the system will follow a predictable and consistent response. For example one would expect the time constant response to a step change in exercise to be invariant over the range of time or work rate i.e. the response to a 'step-up' or on-transient must mirror that of a 'step-down' or off-transient.

This is described in engineering terms as, the output of the response must be predictable when the input into the system and the transfer function are known (fig. 9). The input in this case being work rate and the transfer function being the time constant of the response. The transfer function is the mathematical relationship between the input and output of the response (Wigertz, 1971). In the case of the modelled kinetic response, essentially the only dynamic parameter is the time constant and therefore it can be considered the transfer function. If the relationship $\Delta\dot{V}O_{2(ss)} / \Delta Wk$ in this domain is linear and the time constant invariable then the system can be considered dynamically linear (Fujihara *et al.*, 1973b). The ability to predict the output from an input is the

Boltzmann's principle of superposition (Fujihara *et al.*, 1973a) and the relationship must hold true over an infinite range of time and amplitude variations.

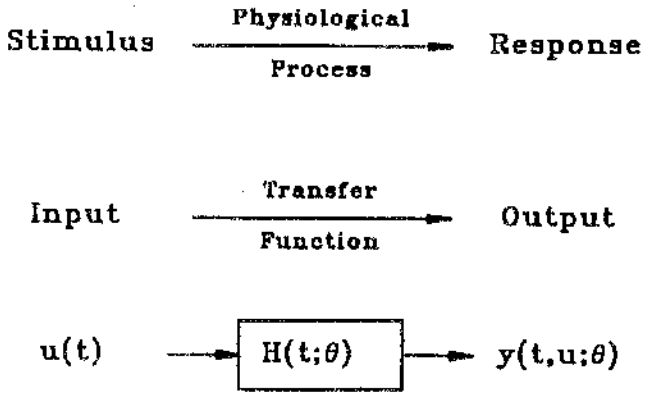


Figure 9 - Schematic system input/output relationship. Assuming that muscle oxygen uptake in the moderate domain is dependent on work rate and time, the transfer function is well described by τ. Therefore the $\dot{V}O_2$ response is calculable for any given work rate stimulus (Lamarra, 1990).

There is little dispute that the steady-state relationship between workrate and $\dot{V}O_{2(ss)}$ is proportional and therefore linear below the lactate threshold. Hill and Lupton (1924) showed in very early work that at low speeds of walking there was a clear linear relationship between walking speed and oxygen uptake (fig. 10). Henry (1951) noted work rate increased linearly at low work rates and that physical conditioning had no effect, a finding confirmed by Whipp and Wasserman (1972). These early findings have been substantiated and as previously mentioned the slope of the $\Delta\dot{V}O_{2(ss)} / \Delta Wk$ relationship has been shown to be approximately 10ml·min·W^{-1} for cycle ergometry (Wasserman and Whipp, 1975; Hansen *et al.*, 1984).

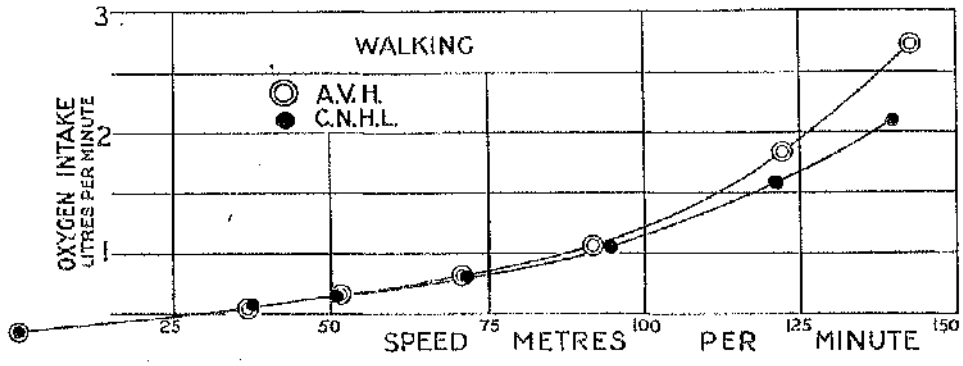


Figure 10 - Graph illustrating the relationship between oxygen uptake and walking speed for two subjects from the early work of Hill and Lupton (1923). Note the linear relationship at lower walking speeds and the non-linear relationship at higher walking speeds. This non-linearity is a manifestation of the 'excess' $\dot{V}O_2$ associated with exercise above the lactate threshold (see text for further details).

The dynamic linearity of $\tau\dot{V}O_2$ is however disputed. On-off symmetry of the $\dot{V}O_2$ response has been demonstrated within the moderate intensity domain (Linnarsson, 1974; Paterson and Whipp, 1991; Ozyner *et al.*, 2001) and is independent of the duration of exercise (Knuttgen, 1970). This is consistent with a dynamic linear response. Furthermore, Cerretelli *et al* (1966), Whipp (1971) and Whipp *et al* (1982) reported the temporal response to a step change in work to be no different from a baseline of rest or light pedalling in this domain. In addition, the kinetic responses to impulse and sinusoidal protocols have reflected dynamic linearity (Casaburi *et al.*, 1977, 1978. Fukuoka and Ikegami, 1990). However these findings are not supported by the work of Hughson *et al* (1988) who reported that temporal $\dot{V}O_2$ responses from step increases in work failed to predict the time course of the response to an impulse protocol i.e. the test of superposition failed (refer to page 22; Fujihara *et al.*, 1973a).

Dynamic linearity has also been questioned with respect to the effect of initial baseline on the subsequent kinetic response. Di Prampero *et al* (1970) and Davies *et al* (1972) reported a faster response when moderate exercise was undertaken from prior exercise compared to that undertaken from rest. In contrast, Diamond *et al* (1977) found no difference in the response from a background of unloaded pedalling. Hughson and Morrisey (1982) and Inman *et al* (1987) reported a slowing of the response when the step change in work rate was initiated from moderate exercise as opposed to rest. These findings were confirmed by Brittain *et al* (2001) who described a slowing of the $\dot{V}O_2$ kinetic response for a moderate work-to-work transition.

The attraction of a dynamically linear first order response is the suggestion of a relatively simplistic mechanism of control. That is control by one fundamental process that remains invariant to challenges in everything but its rate of adaptation. This suggestion of dynamic linearity, while now under challenge, has led many to attempt to elucidate this primary mechanism of control (refer to section 1.11).

1.7 Modelling the $\dot{V}O_2$ response

As described above, the $\dot{V}O_2$ response to moderate square-wave exercise exhibits a three phase response, the second being of a mono-exponential form. The strategy for modelling the $\dot{V}O_2$ response in these three phases depends on what aspect of the underlying physiology is of interest, for example, is it the fundamental phase II kinetics that are of interest or an estimate of the O_2df? There are 3 recognised "models" for fitting an exponential to the temporal $\dot{V}O_2$ response in the moderate domain, figure 11 (Whipp *et al.*, 1982).

If the fundamental kinetics of $\dot{V}O_2$ is the primary interest then "phase" I is excluded from the calculation, with the exponential fit starting after a delay of approximately 15-20 s into exercise. This method of fitting the exponential is termed "model" 3. Discerning the exact point where "phase" I becomes "phase" 2 can be difficult and can be a source of potential error, therefore this should be given careful consideration. Using breath by breath data, suitably averaged from a number of repeat tests (Linnarsson, 1974; Lamarra *et al.*, 1987), the start of "phase" 2 can be estimated as the point after which $\dot{V}O_2$ starts to increase mono-exponentially. In addition, at the "phase"1-"phase"2 transition, the respiratory exchange ratio (RER) begins to decrease due to slower on-transient kinetics of $\dot{V}CO_2$ (τ~ 40-50 s, Whipp *et al.*, 1982) compared to $\dot{V}O_2$ (primarily due to the filling of CO_2 stores in tissues during the early phases of exercise). Furthermore, this abrupt decrease in RER is accompanied by a decrease of PET_{O2} and increase in PET_{CO2} (Cerretelli *et al.*, 1966).

The exponential nature of the phase II kinetic response in theory permits the 'fitting range' to begin at any point along the exponential. Therefore allowing a suitable time delay should prevent any contamination of the phase II response by the phase I response. However in practice it is preferable to use the maximum number of data points for analysis to optimise the confidence of the fit (Lamarra *et al.*, 1987). The distinction between phase I and phase II will therefore be important in protocols designed to assess the magnitude and time course of phase I, or optimise the confidence of a phase II fitting strategy.

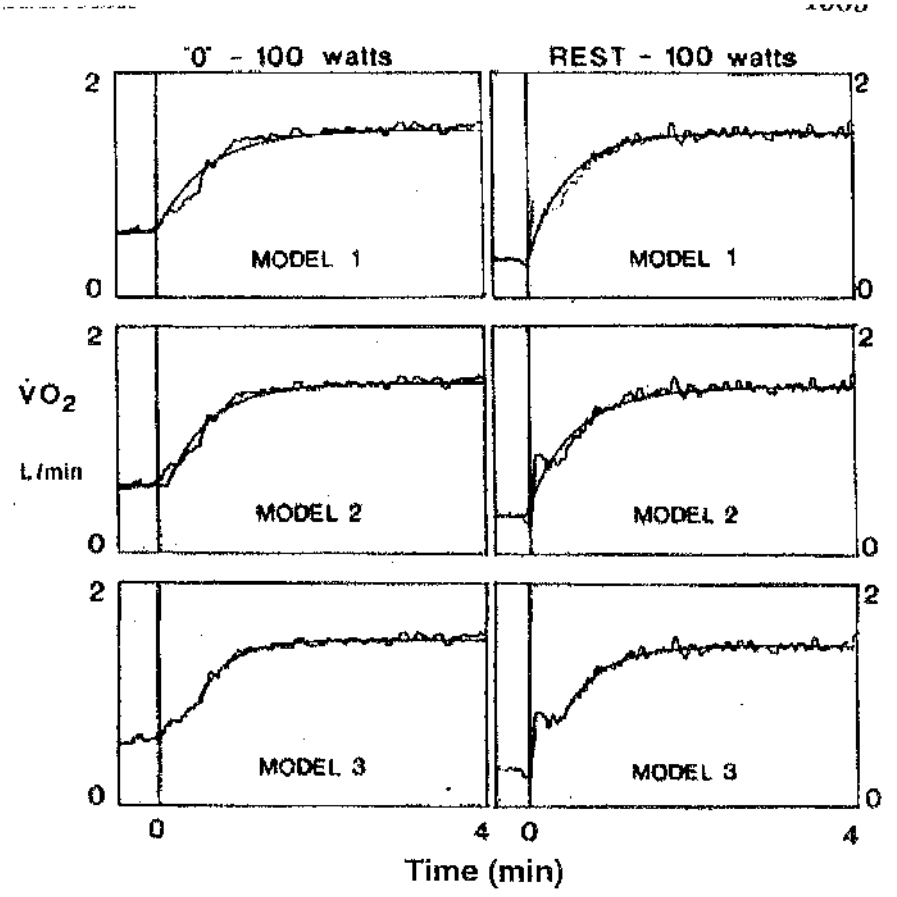


Figure 11 - Best fits of single-exponential models, 1 (top), 2 (middle), and 3 (bottom) to averaged breath-by-breath response of $\dot{V}O_2$ to 100 W exercise from an unloaded baseline (left) and from rest (right). Note that model 3 clearly yields the best fit of the phase 2 response (Whipp *et al.*, 1982).

For calculation of the oxygen deficit both "phase" 1 and "phase" 2 should be included in the exponential fit, as the curve then represents all the non steady-state data. This can be done by either forcing the exponential to start at time zero (start of exercise, "model 1", fig. 11), or by using a best fit exponential through the two phases ("model" 2, fig. 11). "Model" 2 results in a delay period from the start of exercise to the start of the fit. Therefore an adjustment must be made to align the start of the fit with the beginning of the transition. It must be emphasised that the delay term, and τ obtained from the best fit exponential ("model" 2) are purely descriptive and hold no physiological significance. However the same parameters generated from model 1 do have some

credence as they represent a valid index of the total O_2 actually utilised (Whipp *et al.*, 1982).

1.8 Supra-threshold $\dot{V}O_2$ kinetics

Consideration of the $\dot{V}O_2$ response to moderate, square wave exercise is an appropriate frame of reference for further consideration of the response to higher intensity exercise. At work rates above the lactate threshold the $\dot{V}O_2$ kinetic response becomes more complicated (Linnarson, 1974; Whipp and Mahler, 1980; Paterson and Whipp, 1991; Barstow and Mole, 1991; Hughson *et al.*, 1996; Ozyener *et al.*, 2001). Rather than being dominated by a single exponential component, a second slower and delayed component is evident in the response.

1.8.1 'Heavy' exercise

After an initial rapid response (phase I), similar to that for moderate exercise, the early kinetics of the response remain exponential and project towards a steady state gain ($\Delta\dot{V}O_{2(ss)} / \Delta WR$) that is similar to that expected for sub-threshold work rates (Paterson and Whipp, 1992; Barstow and Mole, 1991; Ozyener *et al.*, 2001). Furthermore the time constant for this response is similar to that of sub-threshold exercise (Margaria *et al.*, 1965; Paterson and Whipp, 1992; Ozyener *et al.*, 2001) or slightly slower (Barstow and Mole, 1991). Some 2-3 minutes into the transient a second, slower component is evident in the response (Linnarsson, 1974; Whipp and Wasserman, 1972; Paterson and Whipp, 1992). There are two main consequences of this slow rise in $\dot{V}O_2$ within this domain 1)a necessary delay in attaining a steady-state due to the slow rise of this second component (fig. 12), 2) an increase in the steady-state $\dot{V}O_2$-WR relationship.

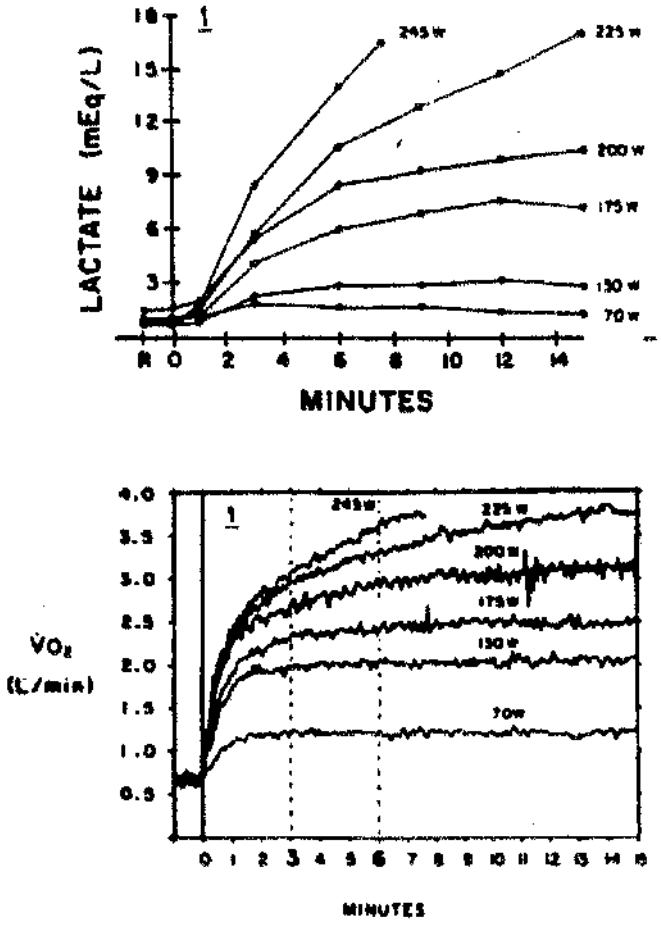


Figure 12 - Temporal profiles of $\dot{V}O_2$ and arterial blood lactate in response to six constant load exercise tests on a cycle ergometer. Test duration was either 15min or to the limit of tolerance. Note that at work rates at which blood lactate increases yet stabilises, steady-state $\dot{V}O_2$ is delayed. At work rates where blood lactate continues to increases, $\dot{V}O_2$ rises to, or toward, a maximum value (Whipp, 1994, modified from Roston *et al.*, 1987).

A time to reach steady-state of 10-15 min is not uncommon in this domain, considerably delayed when compared to a typical time of 3 min for moderate exercise (Wasserman *et al.*, 1967; Poole *et al.*, 1988; Poole *et al.*, 1991; Ozyener *et al.*, 2001). This delay is proportional to work rate within this domain, that is the higher the work rate the longer the time it takes to reach steady state (Henry and DeMoor, 1956; Wasserman *et al.*, 1967; Whipp and Wasserman, 1972; Hagberg, 1978). Roston *et al* (1987) and Whipp (1987) have both reported an increased steady-state gain of ~13 $ml \cdot min^{-1} \cdot W$ (for cycle

ergometry) in this region compared to that of ~ ml·min^{-1}·W for moderate exercise. This 'excess' $\dot{V}O_2$ over and above the steady state requirement for O_2 projected from the sub-threshold $\dot{V}O_2$-WR relationship has been termed $\dot{V}O_{2(XS)}$ (Whipp and Ward, 1993). Some of the proposed theories behind the origin of this excess oxygen requirement are reviewed in section 1.12.

1.8.2 'Very heavy' exercise

As discussed previously (refer to section 1.3.) critical power marks the transition from the heavy domain to the very heavy domain (fig. 3). The early kinetics in this domain once again remain true to the fundamental response described for moderate exercise (Ozyener *et al.*, 2001), with no evidence of the fundamental τ lengthening. The slow component in this domain fails to allow a steady-state to develop and drives $\dot{V}O_2$ rapidly towards attainment of $\dot{V}O_{2\max}$; upon which fatigue soon follows (Ozyener *et al.*, 2001). The greater the work rate the more quickly $\dot{V}O_{2\max}$ is attained (Margaria *et al.*, 1965). Thereby maximal $\dot{V}O_2$ is associated with a range of work rates rather than a unique or 'maximal' work rate (Poole *et al.*, 1988). $\dot{V}O_2$ kinetics in this domain are therefore dominated by the slow component and dictated to in large, by the power time relationship.

1.8.3 'Severe' exercise

The slow and delayed onset of the $\dot{V}O_{2(SC)}$ response has the consequence of little or none of its influence being manifest in the 'severe' exercise domain (fig. 3). The work rates in this domain are those above that associated with maximum WR determined

from a standard incremental cycle ergometry protocol. The result is exercise that may last less than 2 minutes giving the $\dot{V}O_{2(SC)}$ no time for expression (Hill and Stevens, 2001). Therefore the temporal $\dot{V}O_2$ kinetic response in this domain remains a first order mono-exponential rise that, depending on the 'severity' of the work rate, may or may not reach $\dot{V}O_{2\max}$.

1.9 The O_2 Deficit in supra-threshold exercise domains

The mono-exponential $\dot{V}O_2$ response observed in the severe domain allows definition of the O_2 deficit in a similar fashion to sub-threshold exercise. Assuming that the mono-exponential time course still represents a fundamental response, the oxygen deficit can be defined as:

$$O_2df = \dot{V}O_{2\max} \times \tau' \qquad Eqn\ 6.$$

Where τ' is the mean time constant for the response. However the deficit under these conditions is not dependent on the $\Delta\dot{V}O_{2(ss)}$, as in the moderate domain, but on $\dot{V}O_{2\max}$ (fig. 13, panel D).

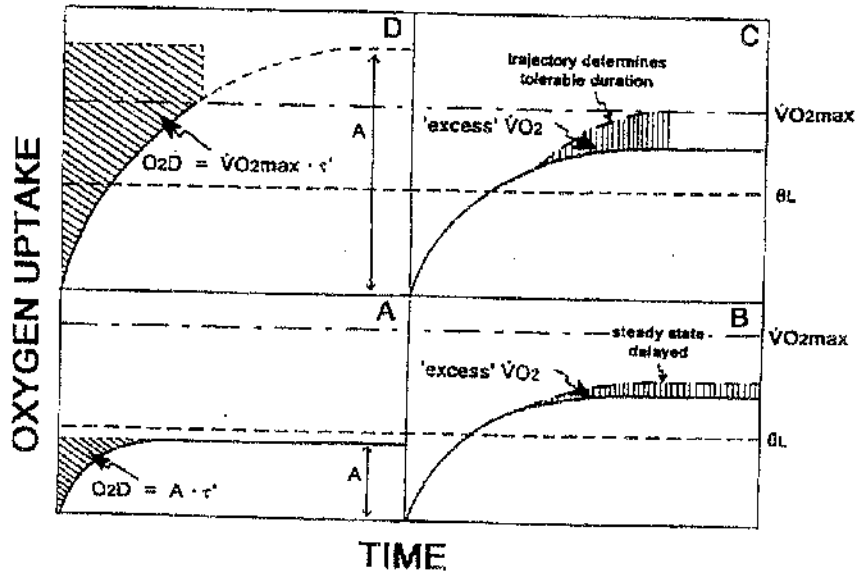


Figure 13 - Schematic representation of the O_2 uptake ($\dot{V}O_2$) response to constant-load exercise at different work intensities. (A) Below the lactate threshold; (B) above the lactate threshold, with $\dot{V}O_2$ reaching a steady-state but with a delayed time course; (C) above the lactate threshold, but with a component of "excess" $\dot{V}O_2$ that leads $\dot{V}O_2$ to attain the maximum $\dot{V}O_2$ ($\dot{V}O_{2\,max}$); and (D) a supramaximal work rate where fatigue occurs so rapidly that the excess $\dot{V}O_2$ component has not had time to develop discernibly. O_2D represents the calculable O_2 deficit. Note that O_2D is not calculable for situations (C) and (B) due to the influence of the slow component, see text for further details (Whipp and Ward, 1993).

As already discussed (refer to section 1.8), the functional significance of the slow component in the 'heavy' and 'very heavy' domains is an increase in steady-state $\Delta\dot{V}O_2 / \Delta Wk$ gain or a prevention of a steady-state altogether. Therefore the asymptote of the $\dot{V}O_2$ response in these domains is not a true reflection of the fundamental response and the O_2 deficit cannot be accurately determined (fig. 13, panels C and B). Therefore the conventional strategy for calculation of the oxygen deficit is not applicable for these work rates. An attempt is often made to estimate the O_2df in this region by extrapolating the sub-threshold $\dot{V}O_2$-WR gain, this is often referred to as the 'accumulated oxygen deficit' (Medbo *et al.*, 1988). This is inaccurate for the reasons described above and the method has recently been shown by Ozyner *et al* (2003) to

result in a negative accumulated oxygen deficit in heavy and very heavy exercise domains.

Bearden and Moffatt (2000) have suggested an alternative strategy for calculation of the O_2 deficit in these domains. They describe a double monoexponential function with separate time delays that characterises both the early $\dot{V}O_2$ response and the slow component independently (fig. 14).

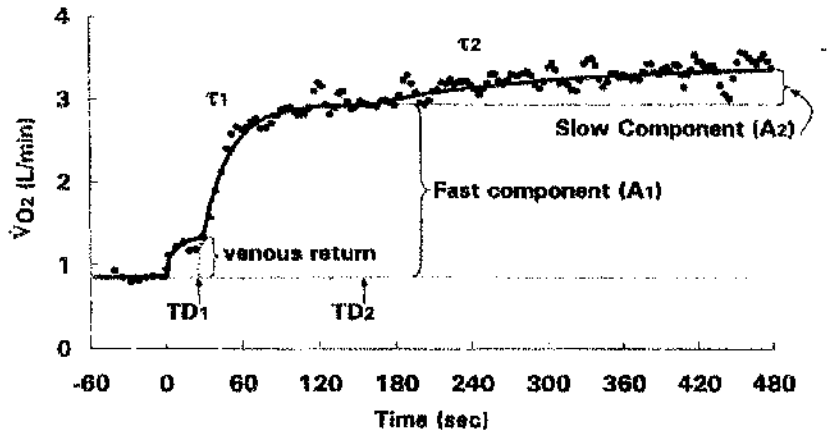


Figure 14 - Graph showing an averaged $\dot{V}O_2$ response to 'heavy' exercise. The model indicates a double exponential function with separate time delays, to characterise both the early $\dot{V}O_2$ response and the slow component independently (Bearden and Moffat, 2000).

They found that areas 1 and 2 (fig. 15) represent a plausible estimation of the O_2 deficit in this domain when compared with post exercise oxygen consumption. This strategy assumes that the relationship between O_2 deficit and O_2 debt above the threshold is the same as that for below the threshold i.e. that the debt represents the oxygen cost of replenishing energy stores 'borrowed' while the system catches up with the demand.

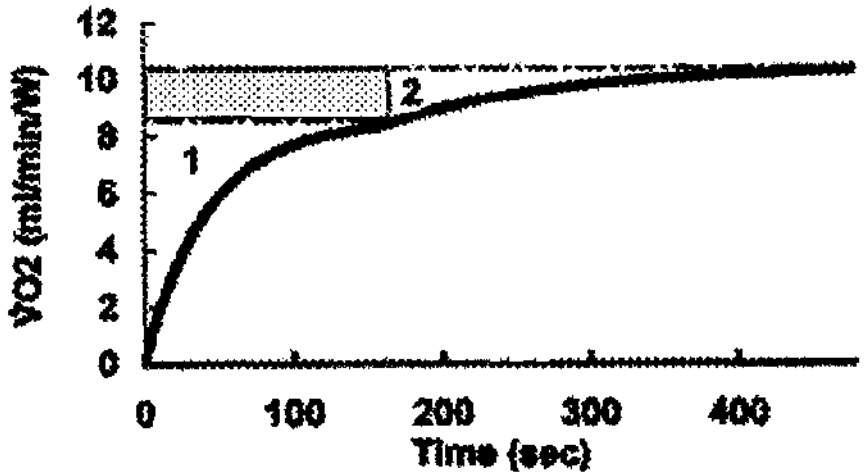


Figure 15 - A schematic diagram of the $\dot{V}O_2$ response to 'heavy' exercise. The fundamental and slow component responses are represented as separate compartments (2-compartment model). Bearden and Moffat, 2000; suggest that areas 1 and 2 represent a plausible estimation of the O_2 deficit in this domain when compared with post exercise oxygen consumption. However the assumption is made that the slow component response is exponential in nature due to it being well fit by an exponential function. This assumption does not always hold true as the projected steady-state for this exponential term is often projected above $\dot{V}O_{2max}$ (Adapted from Bearden and Moffat, 2000).

This same conclusion cannot be made for supra-threshold kinetics until the mechanisms behind the $\dot{V}O_{2(ss)}$ are more fully understood. In addition, the asymptotic value for the slow component in the 'very heavy' domain (fig. 14) will be greater than $\dot{V}O_{2max}$ i.e. A_2 does not represent the projected asymptote of τ_2 (unlike A in panel D fig. 13). A consideration not accounted for in this model.

1.10 The $\dot{V}O_2$ response to incremental exercise

Gas exchange responses to ramp exercise protocols are used extensively because they provide values for the parameters of $\dot{V}O_{2\,max}$, estimated lactate threshold (θ_{LT}) and work efficiency (Wasserman *et al.*, 1994). During a ramped incremental exercise protocol, after a slight delay, $\dot{V}O_2$ increases linearly until a maximum $\dot{V}O_2$ is reached. At all points along this linear increase $\dot{V}O_{2(t)}$ lags behind the $\dot{V}O_2$ steady state response (fig. 16).

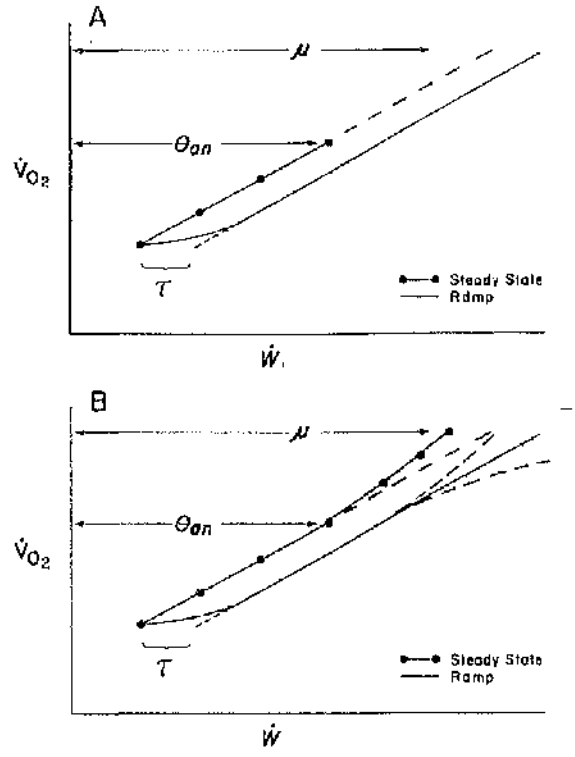


Figure 16 - Schematic representation of the $\dot{V}O_2$ response to incremental work rates during the steady- or quasisteady- state procedure vs the ramp response. The solid lines reflect the actually determined whole-body response. The stippled area below the ramp response represents a possible relative shortage of the O_2 utilisation in the contracting muscles that, when offset by the increased requirements evident on the steady-state test, results in a relatively linear $\dot{V}O_2$ response in the supra- 'anaerobic' threshold (θ_{an}) region. μ represents $\dot{V}O_{2\,max}$ (Whipp, 1987).

This delay is due to the delay in the system response and is equal to the system time constant (τ') (Whipp *et al.*, 1981). Therefore $\Delta\dot{V}O_{2(t)}$ is equal to $\Delta\dot{V}O_{2(ss)}$ at (t-τ'). It is important that this correction is made when calculating steady state work rates based on parameters estimated during an incremental test. The $\dot{V}O_2$ and work rate (WR) established at the upper limit of performance during an incremental test are termed $\dot{V}O_{2\,max}$ and WR_{max} respectively. The influence of the slow component of $\dot{V}O_2$ response is minimal during rapid incremental tests. Only when the increments are of longer duration i.e. 3-4 minutes is the slow component manifest as an upward curvature of the $\dot{V}O_2$ -WR relationship (fig. 16, Whipp and Mahler, 1980 and Hansen *et al.*, 1988).

1.11 Control of sub–threshold $\dot{V}O_2$

The aforementioned first order response in oxygen uptake kinetics to 'moderate' increases in energy demand suggests a single rate-limiting locus of control governing $\dot{Q}_m O_2$. This apparent simplicity in system design has encouraged many to attempt to elucidate this mechanism of control. There are two main theoretical approaches to this subject. The first is that $\dot{V}O_2$ is set by the rate at which oxygen can be transported from atmospheric air to the mitochondria (Hughson, 1990; Hughson *et al.*, 1996; Hughson *et al.*, 1993; MacDonald *et al.*, 1997), the second is that the locus of control lies within the metabolic machinery itself (Cerretelli *et al.*, 1990; Whipp and Mahler, 1980).

Mitochondrial oxidative phosphorylation generates the ATP necessary for the maintenance of muscle cell function over a wide range of metabolic demand. It is the focal point of a close interaction between several metabolic pathways and has the ability to rapidly achieve the necessary rates of ATP production solely through the interaction

of reactants and products (Wilson, 1994). ATP production is so tightly controlled that even at the most extreme demands for ATP, cellular [ATP] is maintained relatively constant (Brown, 1992) and cellular function remains intact (Chance and Williams, 1955).

Mitochondrial respiration utilises the energy from the transfer of reducing equivalents down the electron transport chain to synthesise ATP from ADP and inorganic phosphates (Pi). These reducing equivalents, predominantly NADH but also $FADH_2$, meet oxygen at the terminal electron transport chain complex (cytochrome oxidase) where they are further reduced generating H_2O. The overall reaction is as follows for NADH:

$$NADH + ½\ O_2 + H^+ + 3ADP + 3Pi \leftrightarrow NAD^+ + H_2O + 3ATP$$

Eqn 7.

The value of 3 is proposed by Le Masters *et al* (1984) to best represent the ATP/O stoichiometries of mitochondrial oxidative phosphorylation.

It is probable that control of $\dot{V}O_2$ lies somewhere in the delivery or utilisation of these reactants and products and it seems likely from the uniform concentrations of ATP, during sometimes large swings in demand, that the controlling mechanism is tightly linked to ATP breakdown.

1.11.1 Feedback control through [ADP]

Early theories on the mechanism of control advocated ADP concentration as the primary signal affecting the rate of mitochondrial respiration (Lardy and Wellman, 1952; Chance and Williams, 1955; Chance and Conelly, 1957). Observations using isolated mitochondria showed that respiratory rate increased markedly when ADP concentration was increased (Chance and Williams, 1955), suggesting a simple substrate limitation effect may be regulating ATP synthesis. Furthermore it was calculated that at rest ADP existed at a concentration well below that for its Km, a system requirement for kinetic control (Chance *et al.*, 1986). These observations were consistent with Michaelis-Menton kinetics control of respiration, and the ADP hypothesis assumes that the rate of oxidative phosphorylation is determined by the degree of saturation with ADP following first order (Lardy and Wellman, 1952; Chance and Williams, 1955; Chance and Conelly, 1957; Chance *et al.*, 1985) or second order (Jeneson *et al.*, 1996) control characteristics.

1.11.2 The Adenine Translocase Hypothesis

The adenine translocase hypothesis centres around the transport of adenine nucleotides from site of production to site of utilisation. ATP is synthesised in the mitochondrial matrix, yet is utilised at active sites in the cytoplasm. The reverse is true for ADP. The adenine translocase hypothesis suggests the rate limiting factor in cell respiration is this transport of ADP into the mitochondrial matrix and the transport of ATP into the cytoplasm. Transport is directed by the adenine nucleotide translocase with ATP and ADP exchanged in a 1:1 ratio (Klingenberg, 1980). Pfaff and Klingenberg (1968) first suggested that the rate of extramitochondrial ADP uptake is dependent on

extramitochondrial [ATP] due to competitive inhibition by ATP on ADP transport. Therefore the rate of neucleotide translocation, and mitochondrial respiration, is thought to be dependent on the extramitochondrial [ATP]/[ADP] ratio rather than [ADP] alone (Jacobus *et al.*, 1982).

1.11.3 Feedback control through [Pi]

A principal point of criticism levelled at both the ADP hypothesis and adenine translocase hypothesis is they both exclude a significant role for [Pi]. [Pi] in isolation is thought to be less likely than [ADP] to exert a high degree of control because 31P-NMR spectroscopy measurements *in vivo* have [Pi] at values greater than 1mM, very close to the estimated value for the Km of respiratory control in isolated mitochondria (Chance *et al.*, 1955). It has been shown however that in preparations where [ADP] and [ATP] are kept constant, [Pi] can exert a small but significant effect on respiratory rate in isolated mitochondria and intact cells (Holian *et al.*, 1977; Erecinska *et al.*, 1977).

1.11.4 The 'near equilibrium hypothesis'

The early "near equilibrium hypothesis" advocated a role for [ATP], [ADP] and [Pi] in the mechanism for control (Klingenberg, 1961; Owen and Wilson, 1974; Holian *et al.*, 1977). The apparent Km for ADP has been observed to be dependent on the concentrations of both ATP and Pi (Owen and Wilson, 1974; Holian, 1977). The suggestion is that respiratory rate is more dependent on [ATP]/[ADP] [Pi] ratio, therefore any combination of the 3 components can alter the respiratory rate in response to a change in demand i.e. no single component exerts primary control.

This theory has subsequently been expanded to include a role for mitochondrial reducing power, or the [NADH]/[NAD^+] ratio, through cytochrome c oxidase. The oxygen reduction reaction of cytochrome c oxidase is irreversible (equation 8).

$$2c^{2+} + \tfrac{1}{2}\,O_2 + ADP_c + Pi_c + 2H^+ \rightarrow 2c^{3+} + H_2O + ATP_c$$

Eqn 8.

In the steady state the rate of oxygen reduction by cytochrome oxidase is equal to the overall respiratory rate. Therefore respiratory rate cannot exceed cytochrome oxidase flux, advocating a high probability of cytochrome c oxidase acting as a "rate limiting step". However the supply of the reduced form of cytochrome c is determined by:

$$[c^{2+}] / [c^{3+}] = ([NADH] / [NAD^+])^{1/2} * [ADP].[Pi] / [ATP] * Keq^{1/2}$$

Eqn 9.

Where $[c^{2+}]$ and $[c^{3+}]$ are the reduced and oxidised form of mitochondrial cytochrome c. Therefore the rate of the cytochrome oxidase reaction is linked to the "phosphorylation potential" and provision of reducing equivalents. The balance of equation 9 is near equilibrium-hence the name-and a role for [NADH]/[NAD^+] in driving this reaction has found support (Wilson *et al.*, 1979).

Therefore there are two aspects to control through these interlinked moieties. A demand based hypothesis through [ADP].[Pi]/[ATP] and a supply based hypothesis with the intramitochondrial reducing power as the functioning variable ([NADH] / [NAD^+]). It follows that modulation of either of these factors may influence the rate of respiration.

1.11.5 Gibbs Free Energy

In contrast to, yet also somewhat similar to, the "near equilibrium hypothesis" is the non-equilibrium models based on changes in Gibbs free energy of cytosolic ATP hydrolysis (ΔG_{ATP}) (Kushmerick, 1983; Meyer, 1989; Meyer and Foley, 1994). The two theories are similar in that they are dependent on [ATP], [ADP], [Pi] and [NADH/NAD^+]. Where the non-equilibrium hypothesis differs is that it is independent of kinetic properties of mitochondrial enzymes i.e. it is dependent on the cytoplasmic free energy of ATP hydrolysis or phosphorylation potential (ln[ATP]/[ADP] [Pi]) which is ultimately dependent on the intra mitochondrial [NADH]/[NAD^+] ratio (as described above). Meyer (1988) has advocated a linear model of respiration that describes well thermodynamic control of respiration based on phosphocreatine utilisation. Barstow *et al* (1994a&b) found the non-equilibrium hypothesis to hold during incremental calf exercise (Barstow *et al.*, 1994a), and moderate exercise transitions (Barstow *et al.*, 1994b).

1.11.7 Phosphocreatine breakdown

In response to an increase in work rate there is a mono-exponential decrease in phosphocreatine that has time and amplitude similarities to the oxygen uptake response (Meyer, 1988; Barstow *et* al., 1994a&b; Rossiter *et al.*, 1999). This has been well described in humans by Rossiter *et al* (1999) who used 31P-NMR spectroscopy and breath-by-breath gas exchange simultaneously to make inferences on intramuscular and oxygen uptake parameters respectively (fig. 16).

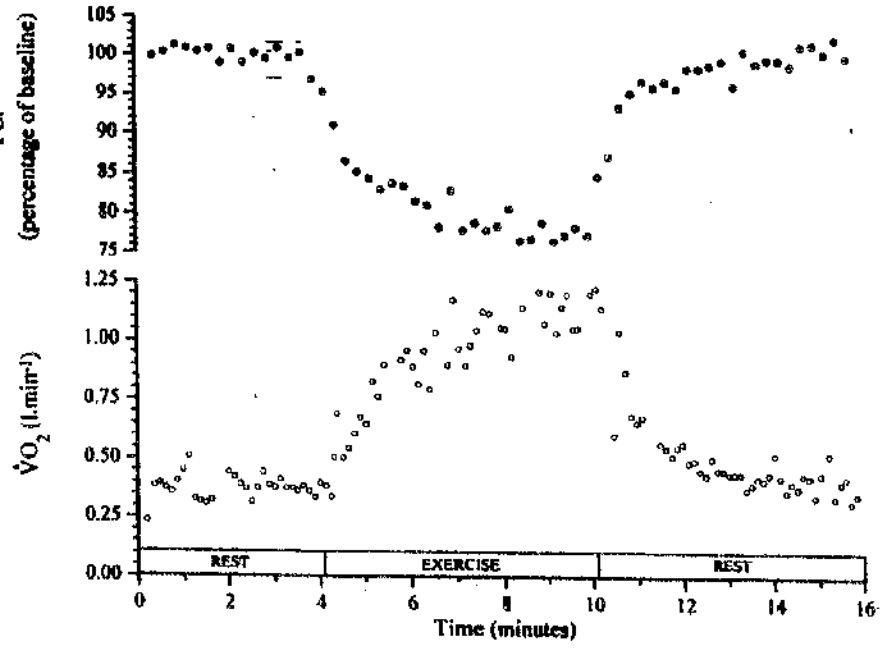


Figure 17 - Simultaneously determined measures of pulmonary O_2 uptake and intramuscular phosphocreatine concentration in response to, and during recovery from, 6 minutes of constant load exercise in a single representative subject (Rossiter *et al.*, 1999).

This apparent co-relationship between [PCr] and $\dot{V}O_2$ has led to speculation that some aspect of PCr degradation exerts a controlling influence on mitochondrial respiration (Mahler, 1985). The basis of this is that the principal controller of the $\dot{V}O_2$ response should exhibit the same time course and magnitude of change as $\dot{V}O_2$ if first order control is to hold (Lamarra, 1990). The "creatine shuttle" hypothesis (Bessman, 1985) suggests that creatine kinase plays a pivotal role in delivering ADP to the ATPases in the mitochondrion. In many ways this is synonymous to the much earlier theories of [ADP] as the rate limiting function. Experimental data (Chance and Williams, 1955) has suggested that, in isolated preparations, the amount of ADP formed is only a few percent of the amount expected. This suggested that the [ADP] arriving at the mitochondria during a twitch may not be sufficient enough for direct control by this

mechanism alone. The proposal from those advocating the "creatine shuttle" theory (Bessman and Fonyo, 1966; Bessman and Geiger, 1981; Mahler, 1985) is that creatine acts as an energy buffer between the sites of ATP synthesis and utilisation dampening the changes in ADP and ATP (fig. 17. Matisone *et al.*, 1996; Kushmerick, 1998). With degradation of PCr into ADP and Cr supplying the required substrate, and subsequent metabolic stimulus to the mitochondria (Jacobus, 1985).

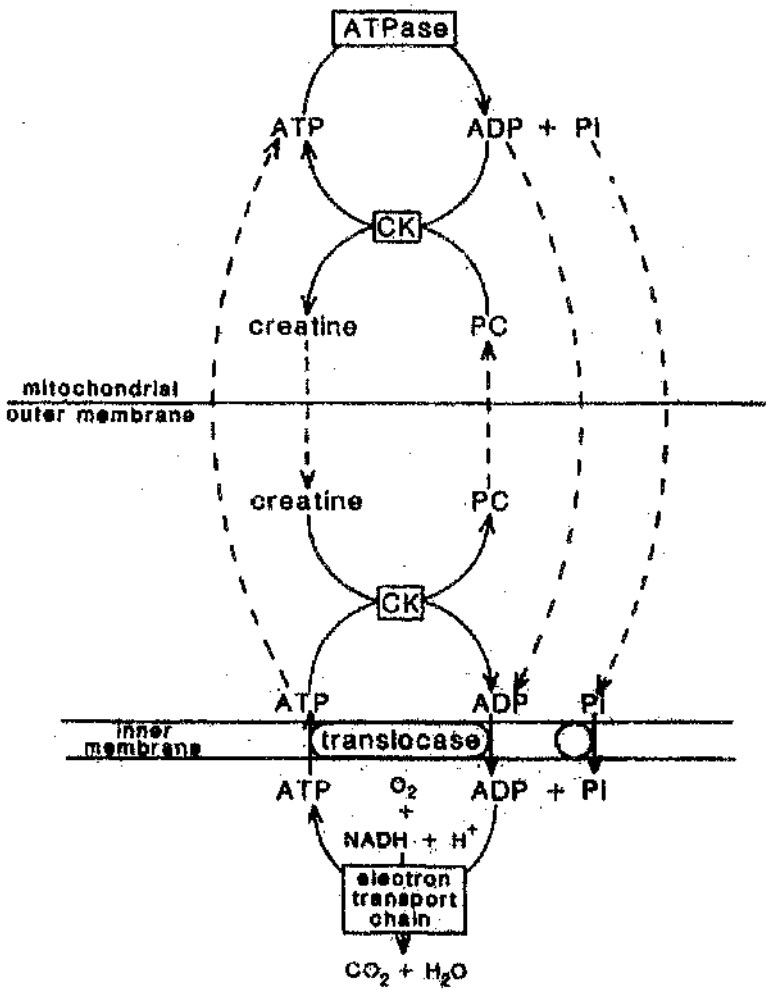


Figure 18 - Simplified schematic diagram of the reactions by which oxygen consumption is coupled to extramitochondrial ATP hydrolysis in muscle. Dashed lines indicate diffusion (Mahler, 1985).

The close proximity of creatine kinase to both the ATP/ADP translocase and crossbridge binding site, lends support to this hypothesis (Bessman and Geiger, 1981); as does the increase in respiratory rate that occurs after ingestion of exogenous creatine (Katz, 1934; Belitzer and Tsybakara, 1939), albeit not proven in intact human muscle (Stroud *et al.*, 1994; Sanz and Marco, 2000; Jones *et al.*, 2002). The results from Rossiter *et al* (1999, 2001, 2002) showing time and amplitude similarities between PCr breakdown and $\dot{V}O_2$ increases below and above the lactate threshold clearly demonstrate a functional link between the two processes. Kushmerick (1998) suggests that locus of control between these two processes is creatine kinase, with feed forward and feedback control not refutable. However feedback control is described better by the models employed and therefore suggested as the more likely.

1.11.8 Oxygen delivery

The role of oxygen as the terminal electron transport chain oxidant puts it in a position as a strong candidate for primary control. The electron generating reactions can only proceed if oxygen is available at the end of the chain to accept protons in the form of hydrogen. It is a requirement then that, to facilitate aerobic resynthesis of ATP, oxygen must be delivered in adequate quantities from atmosphere to mitochondrion. This delivery and utilisation of oxygen has found favour in some quarters as the principal mechanism of respiratory control (Hughson, 1990; Tschakovsky and Hughson, 1999). This thinking has developed largely from observed changes in the time constant for $\dot{V}O_2$ kinetics with increasing or decreasing delivery of oxygen.

Linnarsson (1974) has shown slowing of $\dot{V}O_2$ kinetics with a reduction in the inspired fraction of oxygen, as have Hughson and Kowalchuck (1995). Hughson *et al* (1993) and

MacDonald *et al* (1998) have shown a slowing of $\dot{V}O_2$ kinetics in the supine position compared to upright cycling, findings confirming those by previous authors (Cerretelli *et al.*, 1977; Convertino *et al.*, 1984). This slowing was speeded by the addition of a lower body negative pressure (Hughson *et al.*, 1993) and has been directly related to femoral artery blood flow (MacDonald *et al.*, 1998). Koike *et al* (1990) showed a slowing of the $\dot{V}O_2$ response during moderate exercise when arterial oxygen content was reduced using carboxyhaemoglobin. Similarly Hayashi *et al* (1999) showed that impeding O_2 unloading, through a leftward shift of the O_2 dissociation curve, also caused a slowing of the $\dot{V}O_2$ response. The implication from these studies is that convective or diffusive O_2 delivery affects muscle $\dot{V}O_2$ on-kinetics. However results from MacDonald *et al* (1997), Gerbino *et al* (1996) and the work by Grassi *et al* (1996) suggest this is not the case when 'normal' conditions for O_2 delivery reside.

MacDonald *et al* (1997) demonstrated no change in the time constant for $\dot{V}O_2$, in response to a work load above but not below the lactate threshold while inspiring a hyperoxic gas mixture. This finding was supported by Hughson and Kowalchuck (1995). Gerbino *et al* (1996) demonstrated an apparent speeding of the $\dot{V}O_2$ kinetics above but not below the lactate threshold when exercise was preceded by a 'priming' bout of heavy intensity exercise. The reactive hyperaemia associated with the priming bout did not therefore influence the first order mono-exponential response in the moderate domain. Grassi *et al* (1996) showed, by simultaneous measurement of leg $\dot{V}O_2$, blood flow and arterial-venous oxygen difference ($C(a-\bar{v})O_{2(leg)}$) during sub-threshold cycle ergometery, that blood flow rose immediately on exercise while there was a 10-15 s delay before a rise in $C(a-\bar{v})O_{2(leg)}$. This provides evidence that at the

start of exercise the supply of O_2 was in excess of the demand i.e. muscle oxygen kinetics lag behind the kinetics of bulk O_2 delivery to the muscle.

1.11.9 Mitochondrial Inertia

The lag in muscle oxygen kinetics described by Grassi *et al* (1996) has been proposed to be due to an inherent inertia in the mitochondrial response to an increase in ATP demand. 'Feed forward' control of $\dot{V}O_2$ through 'stock piling' of respiratory substrate has been proposed as the primary mechanism of control (Timmons *et al.*, 1998).

Using the extent of PCr degradation as a direct marker of the oxygen deficit, Timmons *et al* (1998) found it to be reduced by ingestion of dichloroacetate (DCA, a potent stimulator of pyruvate dehydrogenase, PDH). During submaximal (~ 45 % WRmax) single leg extension they found that PCr breakdown and by inference O_2df. was reduced by 50%. DCA increased resting PDH activity threefold, and elevated acetylcarnitine fivefold at rest. The increase in the tricarboxylic acid (TCA) cycle pool size, due to the increased pyruvate flux into the mitochondria, was proposed as rate limiting to TCA flux and to the rate of oxidative phosphorylation (Sahlin *et al.*, 1990).

Howlett *et al* (1999) reported similar results to that of Timmons *et al* (1998). They found that during constant work exercise (~65% $\dot{V}O_{2\,max}$), DCA resulted in reduced phosphocreatine utilization during the first two minutes of exercise. This was accompanied by lower levels of muscle lactate accumulation. Like Timmons *et al* (1998), they found DCA increased both acetyl-CoA and acetylcarnitine at rest; these increased levels were maintained during the first 2 minutes of exercise.

The results from these studies suggested that the provision of substrate is limiting the increase in oxidative phosphorylation at the onset of exercise. Timmons *et al* (1996) termed this the 'acetyl group deficit' and have demonstrated this inherent lag in $\dot{V}O_2$ to be insensitive to oxygen delivery in canine muscle (Timmons *et al.*, 1997). Therefore acetyl-CoA and acetylcarnitine are proposed by Timmons *et al* as the "major determinants" of the oxygen deficit. They hypothesized that on the commencement of exercise, any lag in substrate supply was overcome by the increased acetyl group "store" in the mitochondria. More recently the same group (Roberts *et al.*, 2002) demonstrated this lag in acetyl group provision and PDH activation in the same isolated muscle preparation used by Timmons *et al* (1996).

The results of Parolin *et al* (2000) lend support to this hypothesis. During 15 min of constant load cycle ergometry (55% WR_{max}) in hypoxic conditions, they found that DCA increased PDH activity, acetyl-CoA concentration and acetylcarnitine at rest and at 1 min of exercise. This was accompanied by a reduction in PCr breakdown and blood lactate concentration throughout exercise.

Therefore, for moderate intensity exercise, the data support the notion of an 'acetyl group deficit' with pyruvate dehydrogenase acting as a 'functional stenosis' for oxidative phosphorylation. This is likely to be responsible, at least in part, for the observed lag in $\dot{V}O_2$ at the onset of exercise.

Similarly, Cytochrome oxidase has also been suggested as a site for regulation of mitochondrial inertia at the onset of exercise. Jones *et al* (2003) demonstrated a 19% speeding of the phase II $\dot{V}O_2$ response to moderate exercise when nitric oxide synthase was inhibited. Nitric oxide has been shown to reversibly inhibit mitochondrial respiration, by binding to the O_2 binding site at cytocrome c oxidase in the electron transport chain (Brown, 2000). Using nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase, Jones *et al* (2003) may have reversed this inhibition and augmented the $\dot{V}O_2$ response. The speeding of the phase II response was accompanied by no change in the steady-state gain of the response, a finding supported by the results of Frandsen *et al* (2001) and Radegran and Saltin (1999) who demonstrated no effect of L-NAME on steady-state $\dot{V}O_2$. Kindig *et al* (2002) found the same result in horses during moderate domain running. L-NAME caused a 32% reduction in the time constant of the fundamental $\dot{V}O_2$ response with no change in baseline or steady-state $\dot{V}O_2$. Interestingly this apparent speeding of the $\dot{V}O_2$ kinetics with nitric oxide synthase inhibition occurs from a background of reduced O_2 delivery. Nitric oxide is a potent vasodilator and its inhibition has been shown to reduce cardiac output in the horse (Kindig *et al.*, 2000) and reduce muscle blood flow in rats (Hirai *et al.*, 1994). The results of Jones *et al* (2003) and Kindig *et al* (2002) add evidence to argument against control of $\dot{V}O_2$ kinetics - in the moderate domain - through a limitation in O_2 delivery. The evidence suggests nitiric oxide is an important component in the partial regulation of the temporal $\dot{V}O_2$ response to moderate exercise. Certainly the results of Kindig *et al* (2002) and Jones *et al* (2003) are the first to show a reproducible alteration of the $\dot{V}O_2$ kinetics during whole body dynamic exercise within 'normal' exercising conditions. However, from a functional viewpoint, the efficacy of whole body vasodilator inhibition during exercise remains to be seen.

1.11.10 Summary of putative controllers of $\dot{V}O_2$ in moderate exercise

Factors regulating the control of $\dot{V}O_2$ is a complex conundrum particularly when considering control at the level of the mitochondria. Many factors have been identified that can play a role in the overall $\dot{V}O_2$ response yet their influence and interaction is yet to be fully understood. Brown (1992) has offered an in depth review of the subject from a mitochondria and cell perspective. He suggests the concept is best viewed as a chain of inter linked moieties (fig. 19), that is, the rate of energy metabolism can be controlled at either end of the pathway (or in the middle). Many of the links in the chain illustrated in figure 19 have been summarized here. Also included is the regulation by Ca^{2+} concentration proposed by Hansford (1994).

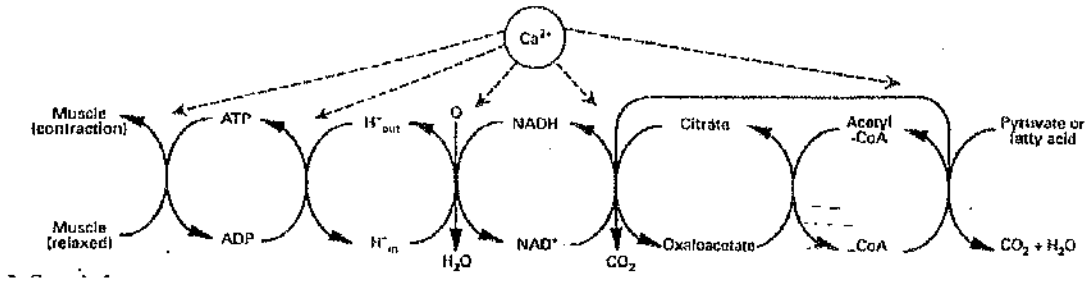


Figure 19 - Schematic diagram illustrating a series of moiety conserved cycles associated with energy metabolism. Brown (1992) suggests that the rate of energy metabolism can be controlled at either end of the pathway or in the middle. The role of calcium in stimulating muscle contraction, matrix dehydrogenases and possible indirectly the synthase and respiratory chains is also shown (from Brown, 1992).

While the studies of isolated mitochondria have given us insight into putative mechanisms of control, they provide limited information as to what governs oxygen uptake in intact human muscle. Many have looked at preparations in a steady-state and/or under controlled conditions that precluded one or more possible co-controllers. The result is often plausible theories that often complement or contradict (Funk, 1990).

Indeed it has been commented (Whipp and Mahler,1980) that many of the studies advocating ADP availability as the principal 'controller', made no attempt to substantiate their models of control by comparing their predicted responses with that observed for intact muscle. Therefore, the 'acetyl group deficit' proposals of Timmons *et al* (1996), the L-NAME work by Jones *et al* (2003) and the *in vivo* $\dot{V}O_2$ / PCr work of Rossiter *et al* (1999, 2001,2002), present the clearest picture of putative controllers of $\dot{V}O_2$ kinetics during whole muscle dynamic exercise. However Brown's concept illustrated in Figure 19 is an important frame of reference when considering this subject.

1.12 Control of Supra-threshold $\dot{V}O_2$: Origin of the $\dot{V}O_2$ slow component.

The principal question surrounding the slow component of the supra-threshold $\dot{V}O_2$ response is whether the 'excess' $\dot{V}O_2$ is representative of an additional oxygen cost of ATP resynthesis in the exercising muscle or an additional oxygen cost of the system response to exercise?

Poole *et al* (1991) revealed (through simultaneous measurement of pulmonary $\dot{V}O_2$ and leg $\dot{V}O_2$), that leg $\dot{V}O_2$ could account for 86% of the rise in pulmonary $\dot{V}O_2$ during cycle ergometry. Rossiter *et al* (2002) confirmed that the exercising muscle is the primary source of the slow component by demonstrating that ~90% of the magnitude of the $\dot{V}O_{2(ss)}$ is reflected within the muscle by its [PCr] response. Both studies suggest there is a link between this 'excess' oxygen cost of exercise and the ATP generating processes in the muscle.

The arterial blood lactate profiles in the supra-threshold exercise domains follow a similar profile to the $\dot{V}O_2$ response. That is; in the heavy domain blood lactate stabilises, in the very heavy domain it continues to rise and in the severe domain it rapidly reaches a maximum (fig. 12). Furthermore the work rate corresponding to critical power also appears to correspond to maximum work rate for blood lactate stabilisation (Poole *et al.*, 1988). Moreover the magnitude of the slow component has been shown to be highly correlated with the rise in blood lactate during exercise (Roston *et al.*, 1987; Poole *et al.*, 1988., Whipp and Wasserman, 1986). Correspondingly, Casaburi *et al* (1987) reported that a reduction in the blood lactate response to exercise, after a period of endurance training, coincided with a reduction in the slow component.

Although this close correlation between lactate and $\dot{V}O_2$ suggests a cause and effect mechanism, this relationship can be dissociated. Roth *et al* (1988) have also shown that circulatory occlusion during exercise, elevating blood [lactate] to a peak of approximately 5 mmol/l has no effect on the post-exercise $\dot{V}O_2$ response. Accordingly Paterson and Whipp (1991) and Ozyener *et al* (2001) demonstrated the on- off- symmetry of the $\dot{V}O_2$ response at work rates where a high post exercise blood lactate concentration would be expected. Additionally, Poole *et al* (1994) elicited lactate concentrations of muscle lactate greater than 9 mmol/l by infusion into dog gastrocnemius muscle without any appreciable effect on $\dot{V}O_2$. Accordingly, the oxygen cost of lactate catabolism during exercise has been shown, in absolute terms, to be small (Bertram *et al.*, 1967; Ryan *et al.*, 1979). Therefore although there is a high correlation between blood [lactate] and $\dot{V}O_{2(ss)}$, in absolute terms the contribution is likely to be small (Whipp, 1987).

One of the favoured proposals regarding the origin of the slow component surrounds the recruitment of Type II muscle fibres. Studies using isolated muscle preparations have shown that Type II fibres require a larger energy cost of force production than type I fibres (Crow and Kushmerick, 1982; Kushmerick *et al.*, 1992). The same findings have been demonstrated with humans (Coyle *et al.*, 1991, 1992; Horowitz *et al.*, 1994; Barstow *et al.*, 1996, 2000). Therefore recruitment of additional type II fibres during heavy exercise would be manifest by an increase in the oxygen cost of the exercise. Support for this theory comes from Barstow *et al* (1996) who reported that the percentage of type II fibres was positively correlated with the magnitude of $\dot{V}O_{2(ss)}$, and Shinohara and Moritani (1992) who demonstrated that the integrated electromyogram (iEMG) of the muscle was positively correlated with the rise in pulmonary $\dot{V}O_2$. Scheuermann *et al* (2001) on critiquing Shinohara and Moritani (1992) suggested that the positive correlation found in their study, which came between the 4th and 7th min of exercise, was after the expected onset of the slow component of 2-3 min (Linnarsson, 1974; Paterson and Whipp, 1992). Furthermore the iEMG did not allow for distinction between which muscle fibre type was being recruited. Scheuermann *et al* (2001, 2002), using an EMG strategy that allowed an estimate of proportional fibre type recruitment, found no evidence of significant recruitment of additional motor units. Therefore they concluded that there is no association between fibre type recruitment and the $\dot{V}O_{2(ss)}$ response during 'heavy' constant load and ramp incremental exercise.

Gerbino *et al* (1996), MacDonald *et al* (1997) and Bohnert *et al* (1998) have shown an apparent speeding of the $\dot{V}O_2$ response for supra-threshold exercise when preceded by a prior "heavy" exercise bout. It was suggested that a reactive hyperaemia from the prior exercise bout may augment the delivery of oxygen to the active muscle fibres increasing

the rate of $\dot{V}O_2$. This provides support for the notion of an oxygen availability limitation to oxidative phosphorylation at these work intensities (Gerbino *et al.*, 1996). These studies employed a mean response analysis for supra-threshold kinetics. Burnley *et al* (2000) using a more descriptive multi-component model to describe the fundamental and slow component responses separately, found that the fundamental response remained unchanged in response to a prior bout of heavy exercise. This suggests that it was the significant reduction in the slow component that better described the results of Gerbino *et al* (1996), MacDonald *et al* (1997) and Bonhert *et al* (1998). Studies by Barstow *et al* (Abstract **), Koppo and Bouckaert (2000) and Scheuermann *et al* (2001) have confirmed the findings of Burnley *et al* (2000). All studies demonstrated a reduction in the slow component with a prior bout of heavy exercise. The original suggestion of a reactive hyperaemia speeding $\dot{V}O_2$ kinetics does not appear therefore to hold true for the fundamental $\dot{V}O_2$ response. This non reliance on O_2 delivery during supra-threshold exercise is supported by the data of Grassi *et al.*, (1998). Using an isolated *in situ* dog gastrocnemius preparation they demonstrated no speeding of $\dot{V}O_2$ kinetics when blood flow was artificially increased, prior to and during an isometric tetanic contraction at 70% $\dot{V}O_{2max}$. Furthermore, Engelen *et al* (1996) showed a slowing of the fundamental response while breathing a hypoxic inspirate but no effect on the slow component response. Bangsbo *et al* (2000) on measuring limb blood flow and oxygen uptake responses during intense knee-extensor exercise, suggested that insufficient oxygen availability is not a limiting factor at the onset of exercise.

Campbell O'Sullivan *et al* (2002) suggest that the lag in the oxidative ATP delivery at the onset of exercise can be over come by a prior bout of exercise. They showed a speeding of the mean $\dot{V}O_2$ response and a decrease in blood lactate and phosphocreatine

utilisation during exercise when exercise was preceded by a prior warm up bout. They suggested that this is evidence of an acetyl group deficit at the onset of exercise, and confirmation of previous work by the same group who reported similar results using dichloroacetate (Timmons *et al.*, 1996, 1998, refer to section 1.11.9). However Savasi *et al* (2002) found no difference in PCr degradation and muscle lactate concentration during 90 s of cycle ergometry at 90% $\dot{V}O_{2\,max}$ upon DCA administration. Grassi *et al* (2002) measured the oxygen uptake response to DCA infusion in isolated canine gastrocnemius muscles to 4 min bouts of electrical stimulation at ~60-70 % $\dot{V}O_{2\,max}$. They also found acetylcarnitine to be increased after DCA infusion, however they found no differences in PCr degradation, muscle lactate accumulation or $\dot{V}O_2$ on-transient kinetics. Furthermore Bangsbo *et al* (2002) directly measured leg $\dot{V}O_2$ during high intensity (~110% thigh $\dot{V}O_{2\,max}$) one legged knee-extensor exercise after DCA infusion. No differences in thigh $\dot{V}O_2$, muscle PCr and muscle lactate production were found (however arterial [lactate] was reduced).

More recently Rossiter *et al* (2003) found DCA had no effect on the time constant of the fundamental $\dot{V}O_2$ and PCr responses during 8 min of high-intensity constant-load quadriceps exercise. However a reduction in the amplitude of the fundamental, and slow component responses was observed. This was accompanied by a reduction in end-exercise lactate, intramuscular acidification and Pi. Therefore neither O_2 availability or mitochondrial inertia at the level of PDH appear to be primary components in the control of the fundamental component or slow component of the supra-threshold $\dot{V}O_2$ response.

Further proposed mechanisms behind the slow component include an increase in circulatory levels of epinephrine (Gaesser *et al.*, 1994), a reduction in unloading of O_2 via the Bohr effect (Wasserman *et al.*, 1991), the O_2 cost of ventilation (Aaron *et al.*, 1992; Carra *et al.*, 2003), the O_2 cost of extraneous muscular work, an increase in temperature (Q10 effect), and a decreased efficiency of P-O coupling subsequent to a temperature rise (Willis and Jackman, 1994). While these mechanisms are plausible their influence on $\dot{V}O_2$ is likely to be limited. Their role in the $\dot{V}O_{2(ss)}$ is likely to be only minor as demonstrated by the on-off-asymmetry in the $\dot{V}O_2$ kinetic response for work rates above the lactate threshold (Patterson and Whipp 1991, Ozyner *et al* 2001). Increases in muscle temperature and the Q10 effect would be expected to be exhibited in the off transient as well as the on transient response.

While the mechanism/s controlling $\dot{V}O_2$ in these exercise domains are unknown it is apparent that the slow component of the response is only evident at work rates which coincide with a sustained increase in blood [lactate] (Roston *et al.*, 1987; Poole *et al.*, 1988; Whipp and Wasserman, 1986), and it is dependent on a relative work rate rather than an absolute work rate (Casaburi *et al.*, 1987). While blood lactate is unlikely to be a primary cause of the $\dot{V}O_2$ excess, these two components of the exercise response appear linked. Studies that have shown a reduction of the slow component with a prior exercise bout have often shown a concomitant decrease in blood lactate (Bonhert, 1998; Gerbino *et al.*, 1996; Barstow *et al.*, 2000). A similar effect has been demonstrated in response to endurance training (Poole *et al.*, 1990; Casaburi *et al.*, 1987; Carter *et al.*, 2000). Therefore some aspect of muscular adaptation to endurance training is an important factor in determining the $\dot{V}O_2$ response to supra threshold exercise. While Sheuerman *et al* (2001, 2002) have suggested that muscle fibre type recruitment during

exercise is not a principal component in the development of the slow component, the predominance of a particular fibre type does appear to have a role. Barstow *et al* (1996, 2000) suggested that the $\dot{V}O_2$ gain ($\Delta\dot{V}O_2 / \Delta Wk$) during ramp and incremental exercise is greater for a high proportion of type I fibres and that the relative contribution of the $\dot{V}O_2$ slow component to the total $\dot{V}O_2$ response to heavy exercise is negatively related to aerobic fitness (as $\dot{V}O_{2\,max}$) and/or the proportion of type I fibres in the working muscles. Furthermore Pringle *et al* (2003) suggest that the time constant for heavy exercise, the slow component of the response, and the fundamental gain are all correlated with Type I muscle fibres. Therefore high aerobic development appears to have an attenuating effect on the slow component but a surprising increase in the $\Delta\dot{V}O_2 / \Delta Wk$ gain and a possible influence on the speed of the kinetic response. The suggested increase $\Delta\dot{V}O_2 / \Delta Wk$ is surprising because fast twitch (type II) muscle fibres have been shown to be less efficient than slow-twitch (type I) fibres (Crow and Kushmerick, 1982; Kushmerick *et al.*, 1992). The findings of Barstow *et al* (1996, 2000) and Pringle *et al* (2003) are similar in some ways to the findings of Koga *et al* (1999) who demonstrated a reduction in the fundamental gain with no change in the $\dot{V}O_2$ time constant and an increase in the $\dot{V}O_2$ slow component when heavy exercise was performed in the supine position. The results of Koga *et al* (1999) are thought to be as a result of a reduced perfusion pressure due to attenuated O_2 delivery, however the similarity of the results may suggest that the fundamental gain in type I muscle fibres is somehow related.

Whipp *et al* (2002) suggest that slow component should not be viewed as a single metabolic compartment with a single τ and a single gain. As described in section 1.9, this does not hold true for models like that of Bearden and Moffat (2000) because of the

inappropriate steady-state value projected from the time course of the response i.e. a steady-state is projected beyond $\dot{V}O_{2max}$. Whipp *et al* (2002) suggest that the slow component is unlikely to represent a single additional metabolic compartment recruited as a square wave at its onset. They propose that it is more likely the $\dot{V}O_2$ response is projecting towards a continually moving asymptote that reflects an additional energy cost of force production within the muscle. This model fits with the on-off-symmetry of the $\dot{V}O_2$ response above threshold and lends itself more to progressive fibre type recruitment throughout exercise rather than fibre type per se as the likely 'primary' factor controlling the time course and extent of $\dot{V}O_{2(ss)}$ expression.

The factors influencing the fundamental $\dot{V}O_2$ response to supra-threshold exercise appear to be associated to those governing the sub-threshold $\dot{V}O_2$ response. However the origin of the $\dot{V}O_2$ slow component remains a mystery. What is clear is that its expression is slow and of delayed onset, and that it is only apparent above the lactate threshold when given time to develop.

1.13 Dichloroacetate

The potential metabolic and clinical properties of Dichloroacetate (DCA) have been a subject of keen interest since the 1960s (Stacpoole *et al.*, 1998); yet due largely to early fears over its toxicity, the vast majority of studies have been confined to the animal world and only a little is known about the extent of its application in health and disease.

DCA is a generic compound formed from, amongst others, the chloridation of drinking water. Previous incarnations include D-gluconodimethylamino acetate or Pangamic

acid (vitamin B_{15}, its earliest form); and di-isopropyl ammonium Dichloroacetate the subsequently discovered, active component, of the early Pangamic acid preparations (Stacpoole, 1989). Stacpoole and Feltz (1970) first discerned the active moiety in the complex was Dichloroacetate itself. It is now more commonly used in a simpler form: Sodium Dichloroacetate.

DCA acts on a number of enzyme processes throughout the body, impacting on the heart, skeletal musculature, liver, kidneys, nerve and brain function. Its main metabolic action is to activate the pyruvate dehydrogenase complex (PDH), lowering levels of blood lactate and increasing pH (Stacpoole, 1989). It is also known to stimulate glycogenesis, inhibit fatty acid oxidation and suppress gluconeogenesis.

The therapeutic value of these metabolic actions has been tested in conditions such as lactic acidosis, diabetes and heart failure, often with positive results (Stacpoole, 1989). Lactate lowering properties have earmarked it as a possible ergogenic aid and its role in activating PDH make it a viable tool for studying metabolic control and response.

1.14 Metabolic actions of DCA.

1.14.1 Activation of Pyruvate Dehydrogenase.

Pyruvate dehydrogenase is a multienzyme complex situated on the inner mitochondrial membrane. Its principal function is to catalyze the irreversible oxidation of pyruvate to acetyl CoA (fig. 20). PDH exists in two forms one inactive, and one active. Regulation of its activity is determined by reversible phosphorolation, with the phosphorylated complex being the inactive form.

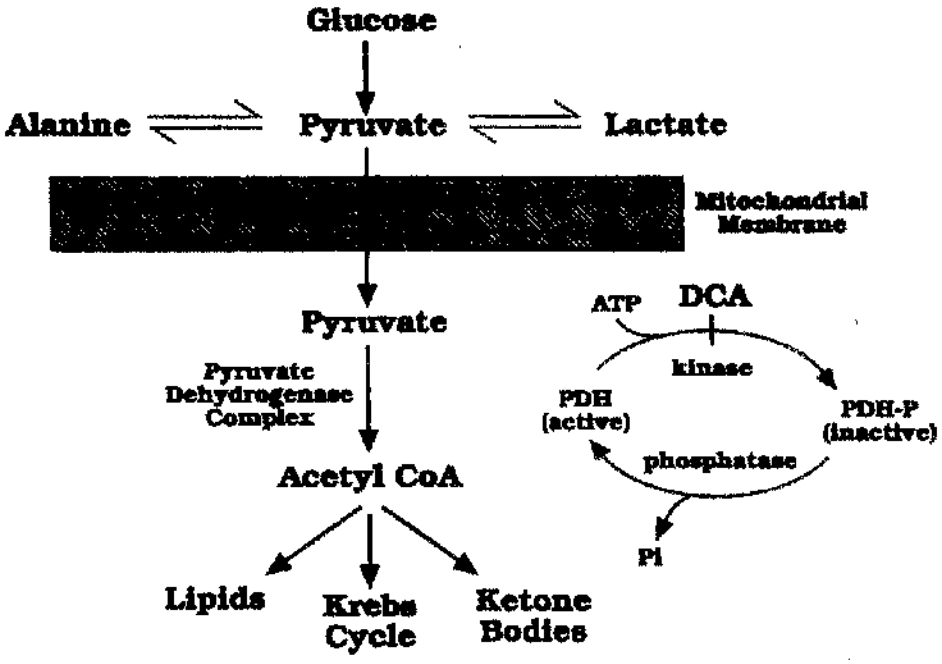


Figure 20 - Site of action of DCA. The PDH component of the pyruvate dehydrogenase complex is reversibly phosphorylated, and inactivated, by PDH kinase. DCA inhibits the kinase, thereby maintaining PDH in its unphosphorylated, active form (from Stacpoole, 1989).

PDH kinase and PDH phosphatase mediate the phosphorylation and dephosphorylation respectively of the PDH complex, Fig. 20 (Stacpoole *et al.*, 1998). DCA inhibits the kinase, preventing rephosphorolation of PDH by ATP and therefore maintains the 'complex' in its active form. Dichloroacetate acts directly on the kinase combining competitive and non-competitive inhibition with ATP. Pratt and Roche (1979) showed that in the presence of ADP the inhibition was competitive, and in the absence of ADP the inhibition was non-competitive. Furthermore, in the absence of ATP and/or calcium, dichloroacetate has no effect (Crabb *et al.*, 1981), and in the presence of acetate the effects can be partially reversed (Putman *et al.*, 1995).

The result of PDH activation is an acceleration of pyruvate flux into the mitochondria, accumulation of available acetyl groups for the tri-carboxylic acid (TCA) cycle, and a decrease in circulating levels of lactate and alanine. As lactate is associated with a hydrogen ion, its reduction results in a rise in pH and production of HCO_3^- (Crabb *et al.*, 1981).

This activation of PDH by DCA is rapid and marked in nearly all mammalian tissues, except the testes and the gut (Stacpoole, 1989). The speed with which DCA exerts its effects (hypolactatemic effects can be detected within 15 min of a single oral dose (Curry *et al.*, 1985; Curry *et al.*, 1991)) is due largely to its rapid uptake by the mitochondria. DCA has been shown to be an excellent substrate for the monocarboxylate transporter of plasma membranes (Stacpoole, 1989). This is the same transporter used by pyruvate, and the two directly compete. In high doses it is therefore possible that DCA may inhibit pyruvate uptake and oxidation by the cell.

Oral doses of DCA (50 mg/kg), lead to plasma concentrations of approximately 1 mmol/l. This is the concentration required for maximal stimulation of enzyme activity in intact cells or tissues (Stacpoole, 1989). The extent to which DCA activates PDH in intact human muscle appears to be approximately 2-4 fold at rest (Timmons *et al.*, 1998; Howlett *et al.*, 1999a&b; Gibala and Saltin, 1999; Parolin *et al.*, 2000).

As already mentioned, hypolactatacmic effects can be detected within 15 min of a single oral dose. This effect reaches a maximum after approximately 60 min, and is sustained for a maximum of 2 h (Curry *et al.*, 1991; Wells *et al.*, 1980). The half-life of DCA in plasma is 30 min- 2 h (Henderson *et al.*, 1997). DCA has an unusual and thought to be unique ability to inhibit its own metabolism (Stacpoole., 1989). An initial dose will

increase the half-life of any subsequent dose. Chu (1987) has found that individuals required from 1 to 8 weeks between identical doses, before the original plasma clearance rate was restored.

The notion of responders and non-responders to dichloroacetate is not one that has been discussed significantly in the literature. It is however an important consideration when interpreting findings. Stacpoole *et al* (1983) noted that when administering DCA to 13 patients with lactic acidosis that only 7 of the patients responded to treatment with DCA. A response was classed as a 20% decrease in arterial lactate concentration. Carraro *et al* (1989) also noted that out of six healthy subjects, PDH activity was not effectively increased in one individual. It is difficult to draw conclusions from the results from Stacpoole *et al* (1983), because the aetiology of the lactic acidaemia in the subject group was diverse, however the results of Carraro *et al* (1989) suggest this is a consideration.

1.14.2 The effect of DCA on lactate metabolism.

DCA has been shown to be effective in significantly reducing lactic acidaemia resulting from genetic predisposition (Stacpoole *et al.*, 1983), acquired clinical conditions (Stacpoole *et al.*, 1983), exercise (Merrill *et al.*, 1980) and hypoxia (Parolin *et al.*, 2000).

Lactic acidosis is a clinical condition caused by the hydrogen ions associated with lactic acid. The aetiology of this disease is diverse. Common causes are diabetes and a genetic deficiency in one or more of the enzymes involved in oxidative metabolism. Typically, blood lactate levels are >5 mmol/l, bicarbonate levels <15 mmol/l, and

arterial pH <7.25 (Park and Arieff, 1982). The mortality in patients with lactic acidosis can be greater than 85% (Park and Arieff 1982). Park and Arieff (1982) have shown in dogs, and Stacpoole *et al* (1983) in humans, that the actions of DCA are enough to significantly reduce lactic acidosis and increase pH.

The successful reduction of blood lactate levels in lactic acidosis has led to the investigation of the effects of DCA in reducing the levels of lactate during exercise. During exercise where there is high anaerobic energy yield, there is a significant production of lactate, and an associated decrease in pH. The production of H^+ ions contributes significantly to the mechanisms of muscle fatigue and muscle soreness during exercise. Therefore reducing lactate concentrations during exercise would be beneficial, especially when considering populations where everyday activity can place significant demands on anaerobic metabolism i.e. heart failure and COPD.

Merrill *et al* (1980) used exercising dogs to study the effect of DCA on circulating lactate *in vivo*. At three workloads of light, moderate and strenuous intensity the rise in lactate was significantly attenuated. A reduction in lactate levels of 60% was reported. Durkot *et al* (1995) using an exercising rat model found lactate levels to be lower after a treadmill run at approximately 60% $\dot{V}O_{2max}$ until exhaustion. Schneider *et al* (1981) again using a rat population, found lactate levels to be reduced during exercise until exhaustion where peak blood lactate was the same in both the DCA and placebo group. A similar finding by Carraro *et al* (1989) in humans, showed that during an incremental increase in work rate DCA decreased lactate concentrations up to approximatly 80% $\dot{V}O_{2max}$. However there was no reduction in the peak lactate concentration at exhaustion. Hatta *et al* (1991) demonstrated a reduction in blood lactate during

submaximal prolonged exercise but no reduction after supra maximal short duration (2-3 min) exercise.

There are two main theories as to why blood lactate concentration increases during exercise. Classically it was thought that a deficiency in the supply of oxygen led to an impairment of respiratory chain function, increasing cytosolic NADH, thereby increasing the reduction of pyruvate to lactate (Wasserman *et al.*, 1973). Alternatively, it has been suggested that increases in exercise intensity result in stimulation of glycolysis, increasing the formation of pyruvate. When the amount of pyruvate exceeds its rate of uptake in to the mitochondria, the rate of lactate production is increased (Stanley *et al.*, 1985).

It may be the case that the reduction of lactate observed at submaximal but not maximal work rates with DCA, is due to a combination of mechanisms resulting in lactate increase. Carraro *et al* (1989), suggested that a limitation in pyruvate dehydrogenase activity contributes to the increase in plasma lactate during submaximal exercise but at maximal intensities their data are consistent with oxygen availability being rate limiting. The dose of DCA they employed was adequate to reduce lactate concentration early post exercise when blood lactate levels were equal to if not greater than that at end exercise. Presuming pyruvate levels remained equally high this would suggest that the DCA dosage was sufficient to lower lactate levels at exhaustion, providing that there was adequate oxygen availability for the maintenance of the respiratory chain function (Carraro *et al.*, 1989). This would suggest that if DCA were to have any effect on exercise performance it would be better employed in longer duration submaximal exercise.

However, in contrast to Carraro *et al* (1989), Ludvik *et al* (1993) using a similar exercise protocol found lactate to be reduced throughout exercise, and Roberts *et al* (1997) found lactate levels to be significantly reduced only between 80 and 100% $\dot{V}O_{2max}$. More recently Howlett *et al* (1999), Savasi *et al* (2002), Grassi *et al* (2002) and Bangsbo *et al* (2002) found no lactate lowering affect of DCA administration in high intensity, short duration exercise (<4 min). Rossiter *et al* (2003) however found end exercise and muscular acidification to be reduced after 8 min of high-intensity knee extensor exercise.

Therefore the effect of DCA on lactate metabolism appears dependent on the intensity and duration of exercise, with an effect more likely to be seen during longer sub-maximal exercise bouts. This may reflect an increase in blood lactate removal by the liver and non-exercising muscles rather than maintenance of heightened pyruvate flux through PDH of the recruited musculature. DCA administration would activate PDH in nearly all mammalian tissues (see section 1.14.1) therefore the balance between lactate production and clearance may favour clearance at lower exercise intensities where production is less rapid.

1.14.3 The effect of DCA on metabolism: general.

In conditions where glycolytic metabolism is not limited DCA appears to have no effect on circulating glucose levels. However DCA is effective in long term reduction of hyperglycaemia in diabetes, and short term reduction of blood glucose levels in the nondiabetic fasted state (Stacpoole and Felts 1970).

The principal mechanism behind this decrease in glucose is stimulation of phosphofructokinase and pyruvate dehydrogenase, increasing glycolysis. The mechanism behind pyruvate dehydrogenase activation has already been discussed (refer to section 1.14).

It is thought that DCA induced inhibition of fatty acid utilisation by muscle may play a part in the regulation of phosphofructokinase activity. It has been proposed that cells exposed to high levels of free fatty acid, such as occuring in diabetes or starvation, oxidise these in preference to glucose (Stacpoole and Felts, 1970). The result would be an increase in the levels of citrate, a known inhibitor of phosphofructokinase. A decrease in fatty acid oxidation has been reported upon administration of DCA (Crabb *et al.*, 1981). Citrate levels should therefore be reduced and glycolysis subsequently stimulated; lowering glucose levels.

The decrease in circulating level of alanine and lactate due to increased pyruvate dehydrogenase activity denies the liver important gluconeogenic precursors. This disruption of the alanine and Cori cycles by which lactate and alanine released from the periphery are utilized for hepatic glucose synthesis, further contributes to a reduced circulating glucose levels upon DCA administration. Diamond *et al* (1982) showed that the effect on hepatic glucose production was due to not only a decrease in the release of alanine and lactate, but also a direct inhibitory effect on hepatic glucose synthesis.

Furthermore DCA has also been shown to inhibit gluconeogenesis from lactate in renal cortical slices from both fed and fasted diabetic and nondiabetic rats (Pratt and Roche, 1979).

At very high concentrations (>10 mmol/l), DCA has an inhibitory effect on fatty acid synthesis. At lower levels (1mmol/l) it has a significant stimulatory effect. The apparent difference between the effect of high and low concentrations is thought to be due to a difference in concentrations in the products of DCA metabolism (glyoxylate or oxalate). Both of which have been linked with an inhibitory effect on the liver, particularly at the level of pyruvate carboxylase (Crabb *et al.*, 1981).

Blackshear *et al* (1974) have reported evidence that suggests DCA inhibits fatty acid oxidation. They proposed that the lowered β-hydroxybutyrate/ acetoacetate ratio and lowered citrate levels in their isolated liver preparations, indicate decreased fatty acid oxidation. Crabb *et al* (1981) suggest that the results indicate more a decreased release of fatty acids rather than an increased extraction.

1.15 The effect of DCA on Haemodynamics.

Stacpoole *et al* (1983) reported that DCA increased systolic pressure and cardiac output in critically ill patients with hypotension and lactic acidosis. This apparent positive inotropic effect occurred before any changes in systolic acid-base status, and was unaccompanied by any changes in heart rate or diastolic blood pressure.

Wargovich *et al* (1988) have shown a positive effect from DCA on cardiac function in Coronary Artery Disease. Left ventricular (LV) stroke volume was increased on administration of 35 mg/kg DCA; as was myocardial efficiency (LV/myocardial oxygen consumption). Heart rate, mean aortic pressure and myocardial oxygen consumption were unchanged. A decrease in systemic vascular resistance was also noted.

Ludvick *et al* (1991), found similar results in healthy volunteers. On administration of a single dose of DCA (50 mg/kg, DCA), cardiac index and oxygen delivery were increased, and peripheral vascular resistance was decreased. No effect was seen in heart rate, oxygen consumption or carbon dioxide output.

The increase in myocardial efficiency reported by Wargovich *et al* (1988) is thought to be due to the inhibition of fatty acid oxidation and stimulation of glucose metabolism. This change in substrate utilisation increases the amount of adenosine triphosphate synthesised per molecule of oxygen. This alternative substrate selection has also been reported in isolated heart preparations (Whitehouse *et al.*, 1974).

This positive effect of DCA on haemodynamic parameters may prove to be of benefit in heart disease where cardiac function is limited, in hypotension, and with exercise intensities where improved perfusion and oxygenation of peripheral tissues may augment the system response to a change in energy demand.

1.16 DCA and exercise performance.

The main factors thought to contribute to muscle fatigue during exercise include [Pi], pH, alterations in Ca^{2+} release and sensitivity, and muscle glycogen content. Of these DCA has been shown to decrease [Pi] and increase pH and elicit a muscle glycogen sparing effect (Timmons *et al.*, 1996; Grassi *et al.*, 2002; Schneider *et al.*, 1981; Durkot *et al.*, 1995; Rossiter *et al.*, 2003). Coupled with a potential speeding of $\dot{V}O_2$ kinetics, these properties have identified DCA as a possible ergogenic aid for exercise performance. This could prove valuable for understanding/improving athletic

performance. More importantly it may prove significant in improving activity levels and quality of life in some patient groups.

An improvement in muscle function upon DCA administration has been reported for isolated muscle preparations. Timmons *et al* (1996) demonstrated that DCA reduced muscle fatigue by ~35% during 20 min of ischaemic contraction in a canine model. Similarly Grassi *et al* (2002) found that the fatigue index in electrically stimulated dog gracilis muscle was improved by ~8% after DCA infusion during a 4 min electrically stimulated isometric tetanic contraction corresponding to ~60-70% of peak $\dot{V}O_2$.

The exercising rat studies by Schneider *et al* (1981) and Durkot *et al* (1995) further support a role for DCA in improving exercise performance. Schneider *et al* (1981) found DCA enabled rats to swim for an additional 99 s before exhaustion. Durkot *et al* (1995) found improved run times in DCA treated rats when compared to controls (169 min vs.101 min). In both studies improved performance was associated with attenuation in the levels of blood and muscle lactate, and an increased efficiency of glucose utilisation.

Hatta *et al* (1991) found that DCA significantly decreased blood lactate concentrations and increased the oxidative removal of lactate during prolonged exercise in mice. They found no effect however, on lactate concentration or removal in supramaximal exercise where the duration of exercise was 2-3 min. The absence of a lactate lowering effect was reflected by equivalence in the exercise duration between both groups. The effect on prolonged exercise duration was not reported.

Ludvik *et al* (1993), Carraro *et al* (1989) and Roberts *et al* (1997) studied the effect of DCA on maximal exercise performance in healthy humans. A positive effect was found by Ludvik *et al* (1993). They reported a decrease in blood lactate concentration throughout exercise, an increase in $\dot{V}O_{2max}$, an increase in maximal work capacity, and an increase in $\dot{V}O_2$ at the lactate threshold (LT). However no effect was found by Carraro *et al* (1989) and Roberts *et al* (1997). Carraro *et al* (1989) found no differences between peak lactate or time to exhaustion during an incremental work protocol, even though blood lactate concentrations were lowered until approximately 80% $\dot{V}O_{2max}$. Roberts *et al* (1997) reported lower lactate concentrations between 80-100% $\dot{V}O_{2max}$. This was not associated with a prolonged time to fatigue or an increase in $\dot{V}O_{2max}$.

Although there is evidence to suggests that DCA has a positive effect on muscle function (Timmons *et al.*, 1996; Grassi *et al.*, 2002; Schneider *et al.*, 1981; Durkot *et al.*, 1995; Rossiter *et al.*, 2003), an improvement in exercise performance has not yet been well described for exercising humans. The research to date suggests that the effects of DCA may be more effective during submaximal rather than maximal exercise. This may be due in part to the relative activity of PDH. In high intensity exercise resulting in exhaustion after ~3 min, PDH activity is only higher during the first minute of exercise as a result of DCA ingestion (Gibala and Krustrup Abstract**). Increased activity during this time period appears to be unable to compensate for the underlying mechanisms of fatigue. During more prolonged exercise DCA can activate PDH above normal levels for a longer period of time (Gibala *et al.*, 1997).

1.17 Toxicology of DCA

There has previously been some concern over administration of DCA to humans, due to potential toxic effects. There have been suggestions that unpurified preparations of DCA were mutagenic. However, studies using DCA of established purity and homogenity failed to show mutagenicity, and early chronic studies using DCA in rodents and dogs revealed no evidence of serious toxicity.

When administered orally for several weeks to Ratsor dogs at doses > 50 mg/kg, DCA is potentially neurotoxic. Peripheral neuropathy has been reported in one young adult man with homozygous familial hypercholesterolaemia who received single daily doses of 50 mg/kg DCA for up to four months. Several weeks after cessation of therapy these changes were resolved. In short term studies with other human subjects no drug related adverse effects have been reported (Stacpoole, 1998).

Therefore, short term studies using "therapeutic" doses 35-100 mg/kg present minimal risk to human subjects. The only potential noticable side effect is a mild sedative, or tranquilizing effect, that in some cases may cause drowsiness for several hours (Stacpoole, 1998).

1.18 The effect of DCA on mitochondrial respiration.

Increased pyruvate flux through PDH as a result of DCA administration has the potential to influence the rate of mitochondrial respiration by increasing substrate availability for the electron transport chain and decreasing the production of lactate associated hydrogen ions.

At the beginning of exercise, energy demand cannot be met solely by aerobic means. In the steady state, the rate of muscular oxidative phosphorylation is able to meet the demands for ATP hydrolysis. During the transition to this steady state however, oxidative phosphorylation is unable to meet the new demand and a fall in ATP is buffered by phosphocreatine (PCr) temporally, until the rate of oxidative phosphorylation can "catch up".

Using the extent of PCr degradation as a direct marker of the oxygen deficit, Timmons *et al* (1998) found it to be reduced by DCA ingestion. During submaximal (~ 45 % WRmax) single leg extension they found that PCr breakdown and by inference O_2def. was reduced by 50%. DCA increased resting PDH activity threefold, and elevated acetylcarnitine fivefold. It was suggested that the increased PDH activity with DCA ingestion, results in an increase in the Tricarboxylic acid (TCA) cycle pool size due to the increased pyruvate flux into the mitochondria. Sahlin *et al* (1988) have proposed that an increase in TCA pool size is rate limiting to TCA flux and to the rate of oxidative phophorylation. The TCA pool (or TCA intermediates (TCAI)), does increase on exercise. However, not all the evidence suggests that this is the principal mechanism behind the results described by Timmons *et al* (1998). Indeed, Gibala and Saltin (1999) noted that TCAI intermediate pool size actually decreased on DCA infusion. They hypothesised that the increased PDH activity at rest, actually diverts pyruvate away from the TCAI pool toward acetyl-CoA formation. Constantin-Teodosiu *et al* (1999) suggested that the TCA pool acted more as a buffer for increased pyruvate availability; and had little significance to TCA flux. This is a notion supported by Gibala *et al* (1998) who observed large changes in TCA cycle flux with relatively small changes in TCAI pool size. In an earlier paper they recommend that TCAI pool size should not be used as an indicator of TCA flux (Gibala *et al.*, 1997).

Howlett *et al* (1999) reported similar results to that of Timmons *et al* (1998). They found that during constant work exercise (~65% $\dot{V}O_{2\,max}$), DCA resulted in reduced phosphocreatine utilisation; measured during the first two minutes of exercise. This was accompanied by lower levels of lactate. Like Timmons *et al* (1998), they found DCA increased both acetyl-CoA and acetylcarnitine at rest; these increased levels were maintained during the first 2 min of exercise.

The results from these studies suggest that the provision of substrate is limiting the increase in oxidative phosphorylation at the onset of exercise. Acetyl-CoA and acetylcarnitine are proposed by Timmons *et al* (1998) as the "major determinants" of the oxygen deficit. They hypothesise that on the commencement of exercise, any lag in substrate supply is overcome by the increased acetyl group "store" in the mitochondria.

1.19 The effect of Dichloroacetate on oxygen uptake.

Evidence from the studies of Timmons *et al* (1998) and Howlett *et al* (1999) indicating a reduction in the O_2 deficit with DCA administration, suggests that there may be a rate limiting step for $\dot{V}O_2$ kinetics at the level of PDH (see section 1.11.9 for further discussion). The apparent reduction in the size of the O_2 deficit would suggest that activation of PDH increases the rate of $\dot{V}O_2$ during the exercise transition. However, evidence from the study of Rossiter *et al* (2003) which directly measured $\dot{V}O_2$ kinetics in response to DCA administration does not support this. Rossiter *et al* (2003) simultaneously measured muscle PCr breakdown and pulmonary oxygen uptake using ^{31}P-magnetic resonance spectrometry and breath-by-breath gas exchange respectively.

A reduction in PCr breakdown was not accompanied by a speeding of the kinetics for $\dot{V}O_2$. The extent to which the kinetics may be speeded through the mechanism suggested by Timmons *et al* (1998) and Howlett *et al* (1999) is not reported. Therefore it is possible that the sensitivity of the $\dot{V}O_2$ measurement in the Rossiter *et al* (2003) study was not great enough to detect a change. This is possible as the metabolic demand of the exercise employed (supine, knee extensor) was low and therefore the $\Delta\dot{V}O_2$ in oxygen uptake small (see section 4.1.2). The results from Howlett & Hogan (2003) support this possibility. They demonstrated an accelerated decrease in PO_2 in isolated single frog muscle fibres with DCA administration, a finding that agrees with the studies of Timmons *et al* (1998) and Howlett *et al* (1999). However, the magnitude of the change (~2 s) is possibly too small to be appreciable *in vivo*.

DCA may also influence the $\dot{V}O_2$ response at work rates above the lactate threshold. As discussed earlier (section 1.12) the temporal response of $\dot{V}O_2$ above the lactate threshold does not follow first order kinetics. An added slow component to the response is observed that delays, or prevents the attainment of a steady-state (Patterson and Whipp, 1991). The slow component is only observed at exercise intensities where there is lactate accumulation in the blood. This association has led to the two responses being linked (see section 1.12). The effect of DCA on lactate production has already been discussed (see section 1.14.2). Its lactate lowering properties during exercise would suggest that any link between the blood lactate response and $\dot{V}O_2$ during supra-threshold exercise could be borne out through DCA supplementation.

A further speeding of the $\dot{V}O_2$ kinetic response may be realized through its reported haemodynamic properties (Ludvik *et al.*, 1991). Gerbino *et al,* (1996) reported that

$\dot{V}O_2$ kinetics above the lactate threshold were speeded by a prior bout of heavy intensity exercise (above LT). The suggested mechanism behind this speeding was an improved muscle perfusion during exercise, as a consequence of a residual metabolic acidaemia. A similar effect may be seen upon DCA administration. Peripheral vasodilatation and increases in cardiac contractility are both reported effects of DCA. These may be significant enough to promote the same form of kinetic speeding observed in the Gerbino *et al* (1996) study. Evidence against this comes from the isolated dog muscle preparations of Timmons *et al* (1996). These investigators reported no changes in muscle perfusion during the DCA trial. However these findings have yet to be demonstrated *in vivo*.

DCA therefore has the potential to affect the $\dot{V}O_2$ response to dynamic exercise. A better description of these responses may identify it as a useful tool for the investigation of possible controllers of the $\dot{V}O_2$ response to exercise. The exact underlying mechanisms behind this response remain to be determined and the actions of DCA may well provide valuable insight. In disease the $\dot{V}O_2$ response to exercise can be dramatically impaired as a result of severe functional limitation. A positive effect on oxygen kinetics through DCA administration may improve such limitations. Identifying it as a possible ergogenic aid for patient groups.

1.20 Objectives and hypothesis

The studies of Timmons *et al* (1998), Howlett *et al* (1999) and Parolin *et al* (2000) all demonstrated a reduced PCr breakdown on DCA administration. By inference, this was suggested as a clear demonstration of a reduced oxygen deficit by either a reduced

steady state gain or a speeding of the kinetic response (Timmons *et al.*, 1988). However none of these studies directly measured $\dot{V}O_2$ and the biopsy frequency for PCr measurement did not allow temporal resolution in any of the studies.

A reduced oxygen deficit may be apparent in a speeding of the $\dot{V}O_2$ on response or a reduction in the fundamental gain component during moderate exercise. If this is the case, a reduced substrate oxidation may also be borne out in an increased 'aerobic range'. This may be evident in a rightward shift of the lactate threshold during incremental exercise, as shown by Ludvik *et al* (1993) but not by Carraro *et al* (1989) and Roberts *et al* (1997).

Therefore the first two studies were designed to look at the effects of DCA on ramped incremental exercise and moderate sub-threshold exercise, using breath-by-breath measurement of $\dot{V}O_2$ for high temporal resolution, blood analysis (lactate, bicarbonate, pH and glucose) and near-infrared spectroscopy as an index of muscle oxygen saturation.

Study 3 was designed to look at the effects of DCA on supra-threshold $\dot{V}O_2$ kinetics using the same measures as above. Rossiter *et al* (2003) most recently showed a reduction in end-exercise lactate, intramuscular acidification and a reduction in the $\dot{V}O_2$ slow component, with no change in the time course of the fundamental response. These effects of DCA may be borne out in an improved exercise performance during prolonged sub-maximal exercise, as found by Schneider *et al* (1981) using rats.

Therefore the aim of this series of experiments is to further investigate the effects of dichloroacetate on oxygen uptake and exercise performance; with a view to understanding more fully the role of pyruvate dehydrogenase as a functional 'stenosis' in the $\dot{V}O_2$ response, and explore the ergogenic effects of DCA on maximal exercise and exercise duration.

The null-hypotheses for these studies is that metabolic actions of DCA do not change the rate or magnitude of increase in $\dot{V}O_2$ during exercise at sub-maximal and maximal work rates.

The alternate hypothesis is that DCA increases the rate and magnitude of the $\dot{V}O_2$ response to sub-maximal and maximal exercise.

Chapter 2

General Methods

2.1 Subjects

Subjects were healthy male volunteers between 18-35 years of age. All procedures were reviewed and approved by the University of Glasgow Research Ethics Committee. The proposals are shown in Appendix 1. After obtaining informed consent all subjects were familiarised with the laboratory set-up and all procedures to a point where they felt entirely comfortable with the protocols and equipment used. A health questionnaire was administered to ascertain health status Appendix 2. Subjects were excluded if they had any history of dizziness, faints, heart or lung disorders, asthma or if they were on any medication or supplement that may have affected heart rate, breathing or exercise performance. All subjects were physically active but not involved in a structured training program or competitive sport.

2.2 Instrumentation

2.2.1 Metabolic Cart

The QP9000 (Morgan Medical, Kent, U.K.) was used for all gas exchange measurements and calculation of metabolic variables. The system comprises of a quadrupole mass spectrometer integrated with a computer and a turbine flow meter. The system allows continuous output of 4 dry gas concentrations and 4 analogue inputs every 20 ms. Results are displayed on-line during the test, saved to disk and were displayed on a chart recorder for continuous hard copy.

2.2.1.2 Mass Spectrometer: Principle of Operation

At the heart of the mass spectrometer operation is the quadrapole. The quadrapole comprises of four precision ground, electrically charged, cylindrical rods arranged concentrically and housed within a vacuum. The quadrupole theory works on the basis that ions of differing mass will behave uniquely when subjected to electrical fields of differing strength.

Measurements are made by drawing gas through a narrow bore sample line at a rate of approximately 50 ml/min. A small portion of this gas is directed into an ion source through the "molecular leak". This is a tiny aperture that lets only a fraction of the gas sample pass through. An ion source bombards the gas molecules with electrons creating ions. These ions are extracted and fed to the quadrupole.

With the appropriate potentials applied to the rods, only the ions of a pre-selected molecular weight range will pass the filter to create a small electrical charge on detecting plates. Therefore, the quadrupole acts as a "tuneable filter" allowing only one species of gas to pass at a time. The rapidity of the analyser allows eight channels, therefore if required eight gases, to be sampled every 20 ms. The electrical signals detected are amplified, digitised and fed to the computer.

2.2.1.3 Flow Sensor: Principles of Operation

A light weight turbine volume measurement module (Interface Associates, Laguna, Nigel, CA.) was used for measurement of expired volume. As the subject breathes, the rotation of a low resistance helical impeller is detected by 4 infra-red light beams

aligned across the flow meter bore. The light beams are broken in sequence as the impeller spins and the breaks in the light beams are detected by phototransistors which generate a voltage output. The number of revolutions per unit time provides a measure of gas flow. Integrated over time this gives volume. The output is linear from 0.005 to 12 l/s with an accuracy of 1.5% (Manufacturer's literature).

2.2.2 Cycle Ergometer

All exercise was performed on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, NL). The ergometer is cadence independent and linear between 40 rpm and 120 rpm (Manufacturer's handbook). Figure 21 shows a Bland/Altman comparison of the output from the ergometer against the input from a Vacumed ergometer calibrator (Vacumed, Model 17800, Ventura, California, USA). The mean difference between the two devices was 4 W over a 20 W - 500 W range.

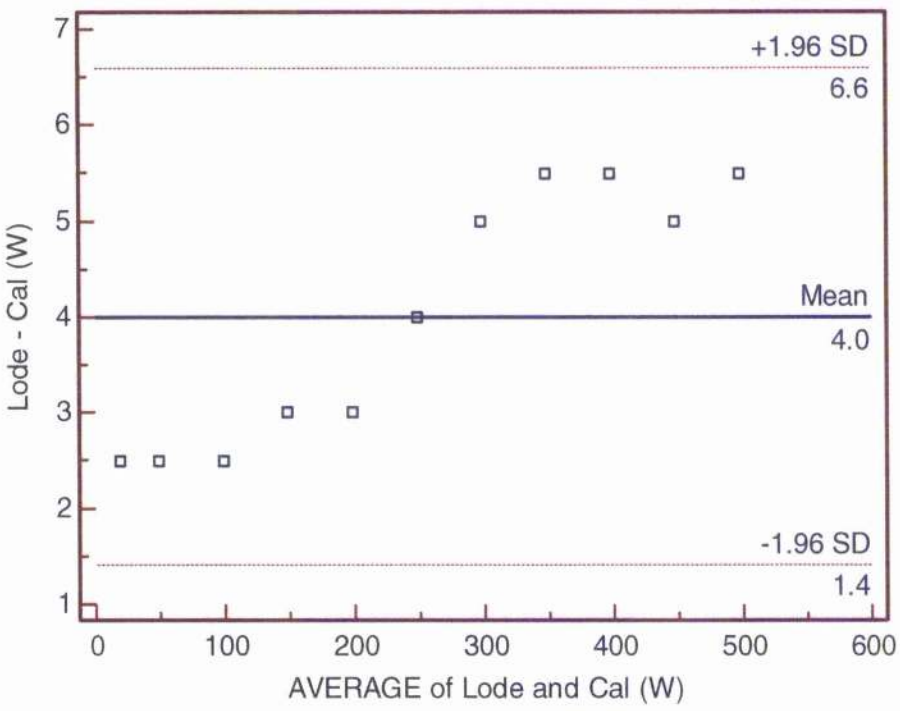


Figure 21 – Biases and limits of agreement for method of measurement plotted against the mean of the reference plus the method (Bland/Altman, 1986). The plot shows a mean bias of 4 W ± 2.6 W (mean ± SD) over a 20 – 500 W range.

The work rate for each test was pre-programmed into a controlling computer interface prior to each test starting. Every attempt was made to ensure that the ergometer was set-up to be as comfortable as possible for each individual. Once a position was established it was measured to ensure the same position was held on each subsequent visit. The addition of triathlon bars allowed the subjects to support their upper body reducing any extraneous work performed by the musculature of the arms, shoulders and torso. When subjects were advised to adhere to a particular range of cadence (RPM), they had the aid of visual feedback in the form of a dial indicating RPM mounted on the front of the ergometer.

2.2.3 Near Infrared Spectroscopy (NIRS)

2.2.3.1 Principles of Operation

NIRS provides a convenient, non-invasive method for investigating the oxygenation status of skeletal muscle. Developed by Millikan (1937) NIRS works on the principle of selective absorbance of near-infrared light (wavelengths between 760 and 1000 nm) by the region of study. When near-infrared light (NIR) is shone at tissue, some is scattered, some is absorbed and some travels through unaffected. The amount of light absorbed is dependent on the oxygenation status of haemoglobin (deoxygenated (Hb), oxygenated (HbO_2), myoglobin (deoxymyoglobin (Mb), oxymyoglobin (MbO_2) and cytochrome oxidase (CtOx). These compounds all contain a strong chromophore group which selectively absorbs NIR light.

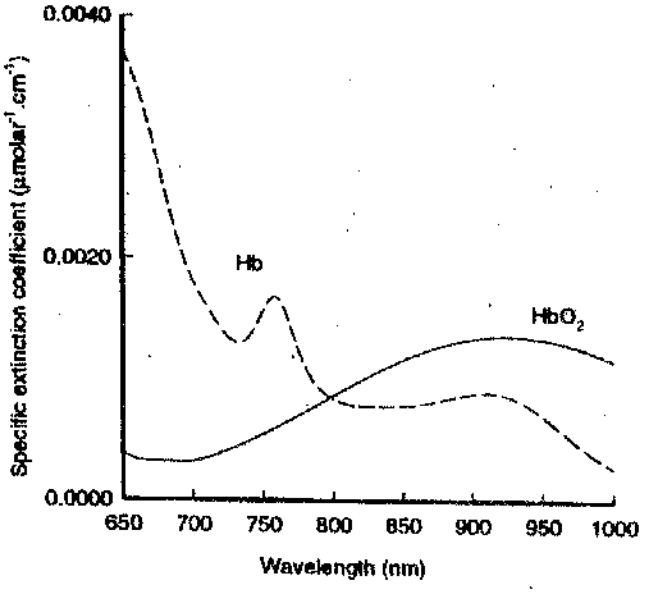


Figure 22 - Diagram illustrating the absorption spectra for Hb & HbO_2 in the NIR region. 650-1000 nm. (Elwell, 1995)

Figure 22 shows the absorbance spectra for Hb and HbO_2 and illustrates the selective absorbance of NIR at different wavelengths. Consequently changes in [Hb] and [HbO_2] in skeletal muscle can be spectroscopically separated (Elwell, 1995). This is described by a modified version of the Beer-Lambert law:

$$\Delta A = \Delta c.\alpha.d.B$$

Eqn 10.

ΔA = change in absorption, Δc = change in concentration, α = specific extinction coefficient (known for each chromophore), d = geometrical distance between light emitter and detector, and B = differential path length factor (constant for a given tissue). A detector picks up the unabsorbed light that passes through the tissue and allows an absolute concentration difference in Hb, HbO_2 and HbT (the sum of Hb and HbO_2), from an arbitrary zero, to be obtained.

It is impossible to distinguish between the contributions of haemoglobin and myoglobin to tissue oxygenation. However the relative contribution of myoglobin compared to haemoglobin has been shown to be small (Mancini *et al,* 1994) and can essentially be assumed to be insignificant with regard to [Hb] and [HbO_2]. Absorbance of NIR by water and lipids in the field of study remains constant and contributes to absorbtion continuously and uniformly (Elwell, 1995).

Differences in the [Hb] signal were assumed to represent oxygen extraction within the muscle since this has been shown to be essentially blood-volume insensitive, in comparison to the [HbO_2] signal which depends significantly on O_2-delivery into the field of interrogation (Ferrari *et al.*, 1997; McCully & Hamaoka, 2000). It might be

expected that the [Hb] and [HbO_2] signals are the reciprocal of one another during exercise and this holds true for exercise in which blood flow is kept constant. However, during dynamic exercise when arterial O_2 delivery and muscle perfusion are not constrained, the anticipated decrease in [HbO_2] as O_2 is extracted is offset by an increase in the measured [HbO_2] as a result of greater HbO_2 delivery into the field of interrogation. Greater HbO_2 delivery, in this case, is primarily due to increased muscle blood flow and perfusion (Kowalchuk *et al*, 2002).

2.2.3.2 NIRS: experimental setup

Intramuscular oxygenation status was measured using a Niro 500 (Hamamatsu) which works on the principles described above. The NIR emitting and receiving optodes were fixed in a light plastic holder that maintained the distance between them at exactly 4 cm. The holder was positioned above the *vastus lateralis muscle* of the right leg and taped to the skin. A wrap of optically dense material was taped around the thigh to prevent contamination of the signal by additional extraneous light. Tape and bandages were used to add further support. The position of the optodes was checked post- test to ensure the optode position remained constant.

2.2.4 Blood sampling, handling and analysis

Arterialised venous blood (Forster *et al*, 1972; McLoughlin *et al*, 1992) was sampled through a 21G venous catheter (Becton Dickinson, Utah, USA), from a dorsal vein on the back of the hand. The venous line was kept clear by a continuous flush of non-heparinised saline (Baxter Healthcare, Norfolk, U.K.) applied through a standard drip. Venous blood was arterialised at rest by incubation for 10 min in a bath of water heated

to 42°C (Forster *et al*, 1972). Throughout exercise the catheterised hand was heated using a variable-intensity heat lamp.

The procedure for taking blood was standardised. The procedure began 15 s before the timepoint the blood sample was to be taken. Waste saline was removed first using a separate syringe. The blood was drawn over 20 s either side of that minute. A 2.5 ml blood sample was taken each time for analysis. The blood was transferred into a container containing EDTA as an anti-coagulant. Once mixed with the EDTA, two 400 µl aliquots were pipetted into two Epindorf tubes containing 800 µl of perchloric acid. Perchloric acid de-natures proteins preventing any further metabolism in the sample of blood. Repeated depressions of the pipette were used to mix the solution. The samples were held on ice, then centrifuged (Hermle Instrumentation Laboratory, Warrington, U.K.) at the earliest opportunity. A small sample of supernatant was analysed immediately for glucose, the rest was transferred to sealable containers and stored at minus 70°C until subsequent analysis for lactate was performed. The lactate samples were run as one batch at the end of each study. This benefited the analysis by ensuring that the same batch of standards and reagents were used for each complete study.

A further 1 ml sample of blood was taken in a heparinised syringe (Radiometer, Copenhagen, Denmark) for analysis of blood gases and pH. The syringes were sealed and stored upright during the protocol for immediate analysis post test.

Blood gases and pH were measured using a blood gas analyser (Synthesis 10, Instrumentation Laboratory, Warrington, U.K.). All samples were analysed within 90 min of sampling. Pilot work from this laboratory has found no variation in blood gas

results comparing samples analysed straight after sampling with those stored on ice for up to 90 min.

Analysis of glucose, lactate and pyruvate were carried out on an automated spectrophotometer (Cobas Mira, ABX, Montpellier, France), using standard methodology (Maughan *et al*, 1982).

The volume of blood taken during all experiments never exceeded 60 ml.

2.2.5 Dichloroacetate: Preparation and dosing

DCA was prepared by pharmacy at The Western Infirmary, Glasgow. The DCA was purchased in a pure form and made up into a 5% solution (5g / 100 ml). This was dispensed as a series of 100 ml bottles. The composition of the solution is listed in table 1. The placebo solution was similar in taste, appearance and consistency (Table 1). All solutions were stored refrigerated (5°C).

Table 1 - Composition of DCA (5%) and Placebo solutions (supplied by Pharmacy at the Western Infirmary Glasgow).

Dichloroacetate Solution	
Dichloroacetate	5 g
Glycerin	20 ml
Sodium Benzoate	100 mg
Aspartame	100 mg
Sodium Acid Phosphate	0.7-53 g
Phosphoric acid	to pK 3.5
Lemon Spirit	0.1 ml
Distilled Water	to 100 ml

Placebo Solution	
Sodium Benzoate	100 mg
Glycerin	20 ml
Aspartame	12.5 mg
Lemon Spirit	0.05 ml
Sodium Chloride	50 mg
Distilled water	to 100 ml

DCA reduces the blood lactate concentration through two mechanisms 1) an increased flux through pyruvate dehydrogenase reducing the amount of pyruvate converted to lactate, 2) a reduction in the rate of glycolysis. For these studies a 50 mg/kg dose was given orally on the morning of the test after an overnight fast. Typically DCA given orally begins to exert a lactate lowering effect after ~15 min (Curry *et al*, 1985; Curry *et al*, 1991), reaching a maximum after approximately 60 min (Curry *et al*, 1991; Wells *et al*, 1980). Thereafter DCA reportedly has a half-life of between 30 and 120 min (Henderson *et al*, 1997). Pilot work showed that our preparation and protocol resulted in a lactate lowering profile similar to that described above (Curry *et al*, 1991; Wells *et al*, 1980). Figure 23 shows the lactate profile over a 2 hour period after a single dose of 50 mg/kg DCA for a representative subject. Based on these results and those in the literature all exercise protocols were started exactly 1 hour after a given dose of DCA or Placebo.

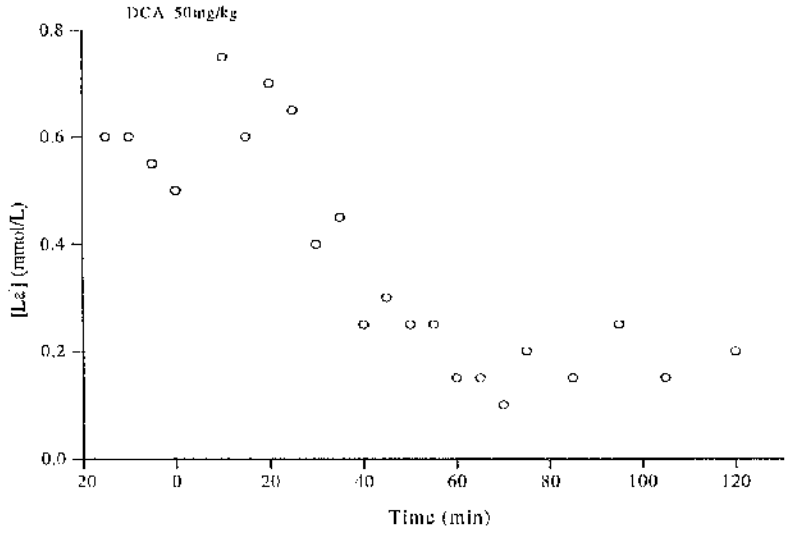


Figure 23 - The effect of DCA administration (50 mg/kg) on resting blood lactate concentration: A representative subject. Blood lactate concentration declines to a minimum at ~ 60 min post DCA ingestion. Thereafter it remains at this reduced level for the further 60 min of data collection, indicating that the DCA preparation is shown to be effective at maintaining a reduced blood lactate concentration during the time period 60-120 min post ingestion. Therefore all exercise protocols were co-ordinated to take place during this period.

Previous studies have employed an intravenous route for the administration of DCA (Wilson *et al,* 1988; Carraro *et al,* 1989; Ludvick *et al,* 1991; Ludvick *et al,* 1993; Timmons *et al,* 1998; Howlett *et al,* 1999). While this was an option as an intravenous line was in place to sample blood, the oral route was chosen so that any problems with the catheter would not preclude the timing and administration of the DCA. Stacpoole (1998) has reported that, although the peak plasma drug concentrations occur immediately after intravenous administration, the bioavailability of DCA after administration by both methods is similar.

None of the subjects reported any discomfort or side affects after DCA administration.

2.2.6 Calibration

2.2.6.1 Mass spectrometer and flow sensor

Calibration of both the mass spectrometer and the flowhead were carried out prior to each test. The mass spectrometer is calibrated using a precision analysed gas of known concentration (BOC Gases, Guildford, Surrey, U.K.). The calibration gas is passed through the system producing a series of peaks at points on the mass spectrum corresponding to the relevant masses of nitrogen (28), oxygen (32) and carbon dioxide (44). The system tunes each channel to the apex of the peak and applies the gas concentrations entered to calculate a gain that passes through the origin. The calibration is checked by passing two high-precision analysed gas mixtures through the system to provide representative sets of values. This was repeated before and after each experiment. If drift of more than 1% was encountered in the measured gas values then the results from the experiment were disregarded.

The volume measurement module or flow head was calibrated using a precision 3 litre syringe (Hans Rudolph, Kansas City, MO, USA). This volume was passed 10 times through the system to produce repeated measures for inspiratory and expiratory volumes. The mean volumes were compared with the known volume and the calibration was accepted if they differed by no more than 0.2%.

2.2.6.2 Transit delay

The raw signals for br x br volume and gas composition are separated by the time it takes the gas sample to reach the analyser (the volume signal is electrical and therefore almost instantaneous). This time difference is termed the transit delay. Calculation of the transit delay ensures that both signals are appropriately time aligned. This is achieved by incorporation the result into the br x br algorithm (refer to section 2.2.7).

The delay time was established before each test. To do so a bolus of gas was introduced into the breathing valve apparatus manually using a syringe filled with expired air (Beaver *et al*, 1973). The computer detects the rise of the flow and CO_2 signals. These signals are then time aligned and the calculated time difference is offered for acceptance to the operator. The procedure is repeated 3 times to check the result is repeatable before the value is accepted, and automatically incorporated into the gas exchange computations. Normal transit delay time was approximately 500 ms.

2.2.6.4 Near infrared spectroscopy

The NIRO 500 was calibrated using a standardised maximal voluntary contraction (MVC) of the right quadriceps (Chance *et al*, 1992; Belardinelli *et al*, 1995). To do this the subjects assumed a seated position with the right leg at an angle of 90°. The subjects achieved a contraction by pushing down through their foot. The contraction was maintained until the [ΔHb] signal reached a maximum. The duration of the manoeuvre was typically 30 s.

The MVC manoeuvre allowed maximal values for [ΔHb], [ΔHbO_2] and [ΔHbT] to be determined. These values were used to express the data as a percentage of maximum, normalised to a zero baseline (Chance *et al*, 1992; Belardinelli *et al*, 1995). Therefore the MVC manoeuvre and the subsequent data correction act as a physiological calibration of the system. The alternative to a maximal contraction was inflation of a pressurised cuff around the proximal girth of the quadriceps (Mancini *et al*, 1994). However pilot work showed this method to be less effective. Figure 24 shows clearly two repeated MVC measurements followed by a less saturated cuff measurement during cuff inflation to 180 mm Hg. The use of maximal exercise to induce complete O_2 extraction is supported by Chance *et al* (1992), who demonstrated no further intramuscular desaturation when ischaemia was induced by an inflatable cuff at the point of exhaustion. On this basis the MVC manoeuvre was preferred as the method of calibration. However it was decided that the MVC should be performed post test as performing the manoeuvre pre test may effect DCA metabolism. On the familiarisation visits the MVC was performed pre and post test to assess the affect of the exercise protocol on the maximal contraction. No differences were found between pre and post MVC values.

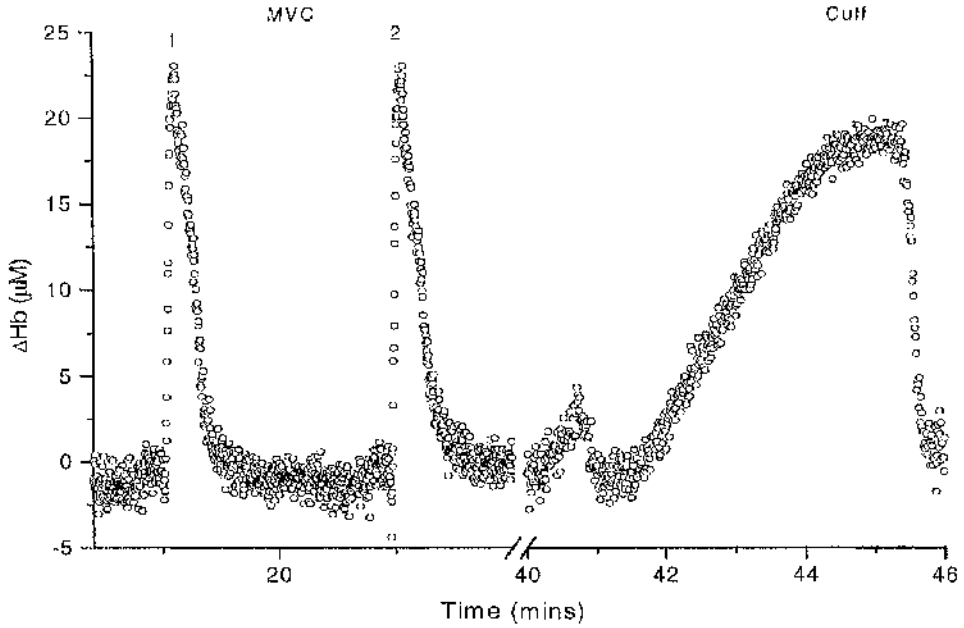


Figure 24. - A comparison of the maximal voluntary contraction (MVC) manoeuvre for NIRS calibration, and measurements taken upon inflation of a high-pressure inflatable cuff. The MVC manoeuvre is shown to be repeatable in accordance with previously published results (Chance *et al*, 1992; Belardinelli *et al*, 1995). An alternative to the MVC manoeuvre is inflation of a high pressure cuff around the proximal length of the quadriceps (Mancini *et al*, 1994). This methodology lessens the metabolic impact in comparison to the MVC manoeuvre. However, the maximal level of Hb desaturation achieved through this method was not as great as that achieved through the MVC manoeuvre. Therefore, an MVC calibration of the NIRS setup was performed post exercise to avoid impacting on the level of PDH activation prior to exercise.

2.2.7 Gas exchange algorithm

Gas exchange in the lung is calculated using algorithms based on those of Beaver *et al* (1973) from the measurements of fractional gas concentrations and expired volume. Beaver *et al* developed the algorithm to calculate gas exchange over a breath (defined: beginning of inspiration to the end of expiration), correcting for the dead space of the breathing apparatus, water vapour condensation, changes in lung gas stores and a phase difference between the generated flow and gas signals (delay time). What follows is a simplified summary of their rationale to illustrate the principle behind calculation of $\dot{V}O_2$ over a single breath.

The total volume of oxygen is equal to the volume of inspired oxygen minus the volume of expired oxygen i.e.

$$\dot{V}O_2 = \dot{V}_I O_2 - \dot{V}_E O_2$$

Eqn 11.

Where $\dot{V}_I O_2$ is inspired volume of O_2, and $\dot{V}_E O_2$ is expired volume of O_2. It follows that:

$$\dot{V}O_2 = \int_{insp} \dot{V}I dt \cdot F_I O_2 - \int_{\exp} \dot{V}E dt \cdot F_E O_2$$

Eqn 12.

$\dot{V}I$ is inspired volume, $\dot{V}E$ is expired volume and F_IO_2 and F_EO_2 are fractional inspired and expired oxygen content respectively.

The computation of $\dot{V}O_2$ is based on the measurement of expired volume. The assumption is made that there is no net transport of nitrogen across the lung; and that any changes in nitrogen concentration measured occurs from changes in lung gas stores i.e. changes in end expiratory lung volume and/or the composition of the lung gas stores.

Therefore the term for inspired volume in equation 13 is compensated for by the assumption that there is no net exchange of inert gases, principally nitrogen, in the lung. Thus:

$$\dot{V}I \times F_I N_2 = \dot{V}E \times F_E N_2$$

Eqn 13.

Therefore

$$\dot{V}I = (FEN_2 / FIN_2) \times \dot{V}E$$

Eqn 14.

Where FIN_2 is the fraction of inspired nitrogen and FEN_2 is the fraction of expired nitrogen. Argon is compensated for in the calculations by normalising the sum of the composite gases to 99.07% i.e. assuming argon has a constant atmospheric value of 0.93% and $FIN_2 + FIO_2 + FICO_2 = 0.9907$.

It follows that

$$\dot{V}I = \frac{(1 - FEO_{2} - FECO_{2})}{(1 - FIO_{2} - FICO_{2})} \times \dot{V}E$$

Eqn 15.

Therefore equation 13 becomes:

$$\dot{V}O_{2} = \left[\frac{FIO_{2} \times (1 - FEO_{2} - FECO_{2})}{(1 - FIO_{2} - FICO_{2})} - FEO_{2} \right] \times \dot{V}E$$

Eqn 16.

The same principle is applied to calculation of CO_2 output, except that expiration and inspiration are reversed in the calculation as the net direction of CO_2 flow is out of the lung. The algorithms of Beaver *et al* (1973) go further than this simplified derivation to take into account the effect of breathing valve dead space (i.e. compensating for a small, initial volume of the inspired gas having an expired gas composition), water vapour dilution and temperature (expired gas is normally assumed to be fully saturated at body temperature at the site of sampling). Prior to the start of every test, values for barometric pressure, ambient temperature, ambient humidity and the mass spectrometer delay time were entered into the computer.

2.2.8 Breath-to-breath "noise", editing, and analysis of breath-by-breath data

Breathing has inherent irregularities that produce breath-to-breath fluctuations in gas exchange or "noise". The confidence with which one can estimate a parameter depends largely on how noisy the data set is; i.e. the more noisy are the data, the lower will be the confidence in the value estimated for the parameter (Lamarra *et al*, 1987). Lamarra *et al* (1987) demonstrated that this breath-to-breath "noise" follows an uncorrelated Gaussian stochastic process, the standard deviation of which is largely independent of metabolic rate.

These authors calculated that the expected confidence limits (K_n) for the estimation of the time constant of $\dot{V}O_2$ was characterised by the equation:

$$Kn = \frac{L \cdot So}{\Delta n \cdot \Delta \dot{V}O_{2ss}}$$

Eqn 17.

L is a constant, the magnitude of which is dependent on the underlying time constant of the $\dot{V}O_2$ response. It represents the amount of data relevant to the estimation i.e. the longer a time constant the more data points/time points there are available for analysis. So is, the standard deviation of the breath-to-breath fluctuation in $\dot{V}O_2$ and n the number of transitions that have been time-aligned and superimposed. Therefore, for any time constant calculated, the estimated value will be at best within the value K_n.

Based on the work of Lamarra *et al* (1987), removal of breaths outwith four standard deviations of the mean was carried out as standard on all raw breath-by-breath data. This procedure is statistically justified because small-sample physiological data appear to contain such values more frequently than expected from a Gaussian distribution, and retaining such values may degrade the results from responses containing limited amounts of data (Lamarra *et al*, 1987). Further editing of breaths was strictly kept to removal of breaths that were physiologically atypical i.e. resulting from coughing, sneezing or swallowing.

Where applicable the breath-by-breath data was then interpolated to 1 s intervals allowing repeated tests to be averaged by superposition (Whipp *et al.*, 1982); i.e. taking account of variations in breath duration (fig. 25). Following superposition, the resulting ensemble-averaged response was then further averaged over 8-12 s to provide an average $\dot{V}O_2$ response suitable for kinetic analysis (i.e. with more favourable signal-to-noise characteristics).

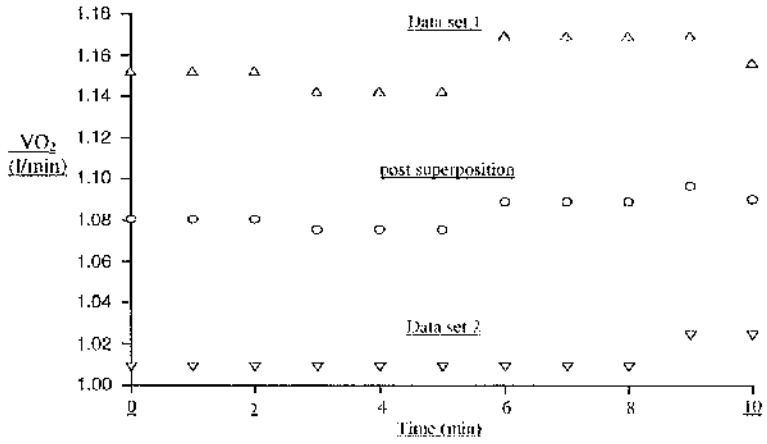


Figure 2.5 - The effect of the superposition process on a data set. The open triangles represent the actual collected data. The open circles represent the data after the superposition process. Superposition allows data from a number of repeat collections to be 'grouped' for analysis. This process reduces the breath-to-breath 'noise' associated with breath-by-breath data. Thereby improving the accuracy with which a model can be fit to the data (Lamarra *et al*, 1987).

2.2.9 Ratings of perceived exertion and dyspnoea

A 0 – 100 % visual scale was used to determine ratings of perceived exertion and ratings of dyspnoea. The scale consisted of a row of 100 LED lights that could be lit in sequence using a dial mounted on the handle bars of the ergometer. Subjects were asked to refer to the scale and illuminate the light strip to the appropriate score for ratings of perceived exertion and ratings of dyspnoea in turn. The score was noted and the scale reset to zero between each rating.

2.2.10 General Constraints

The major constraint in these studies was the use of dichloroacetate. While there have been no significant side effects reported with acute doses of the drug, it is still recommended that doses be kept to a minimum (personal correspondence, Dr P.W. Stacpoole and Dr Y. Pitsiladis). The maximal single dose in previous human studies has not exceeded 100 mg/kg body weight. The sum of the doses employed in studies with repeated dosing regimes has not exceeded this maximum when the population under study has been healthy (Roberts *et al.*, 1997; Ludvik *et al.*, 1991; Ludvik *et al.*, 1993; Timmons *et al.*, 1998; Carraro *et al.*, 1989; Howlett *et al.*, 1999; Wilson *et al.*, 1988; Rossiter *et al.*, 2003). A lactate-lowering effect has been shown with doses ranging from 20-100 mg/kg, the effect being more pronounced with higher doses. An oral dose of 50 mg/kg was chosen for these studies. This dose had been used previously in human studies (Roberts *et al.*, 1997; Ludvik *et al.*, 1991; Ludvik *et al.*, 1993; Timmons *et al.*, 1998; Rossiter *et al.*, 2003) allowing a relevant comparison of results. A lower dose than the theoretical maximum of 100 mg/kg would also allow a repeated dose if a subject volunteered to take part in more than one experiment. Pilot work (refer to fig 23), showed that the dosing strategy reduced resting blood lactate by >50 %.

It is preferable to repeat the experimental protocol and add the data to the previous data set (*Eqn.* 17) to further enhance the accuracy of a fitting strategy. As indicated above, this is often applied to breath-by-breath gas exchange data, especially when kinetics is the focus of interest. In practical terms many repeats of the same exercise protocol are performed within the same day or over a number of days. However, the constraints of DCA usage limited us greatly in the number of repeats that could be performed and therefore resulted in a reduced confidence of parameter estimation.

2.2.10 Statistics

Statistical analysis was performed using Minitab (version 13.1, Minitab Inc., State College, Pennsylvania) and Microsoft Excel (Microsoft Corporation). The statistical approach was taken after consultation with an independent statistician.

Data sets were tested for normality using Anderson-Darling normality tests, and, where necessary, data were logarithmically transformed before statistical analysis. Statistical analysis was carried out using two-factor ANOVA with repeated measures, followed by Student's paired *t*-test if a main treatment effect (i.e. placebo vs. supplementation) was observed. Statistical significance was declared at $P<0.05$. Data are presented as mean values ± SEM unless otherwise stated.

The $\dot{V}O_2$ modelling functions were fit to a non-linear least-squares regression using commercially available software (Microcal Origin). The 'goodness' of fit was determined by observing the flatness of the associated residuals and minimising the sum squared errors.

Chapter 3

The effect of dichloroacetate on oxygen uptake during incremental exercise

Chapter 3

3.0 Introduction

The studies of Timmons *et al* (1998), Howlett *et al* (1999) and Parolin *et al* (2000) reported a reduced PCr breakdown during exercise after DCA administration. This led to the inference of a reduction of the oxygen deficit due to an acceleration of oxidative phosphorylation from the start of exercise and/or a reduction in the fundamental gain. The associated reductions in blood lactate concentration further suggested that DCA augmented the aerobic capability of the exercising muscle (Merill *et al.*, 1980; Durkot *et al.*, 1995; Schneider *et al.*, 1981; Carraro *et al.*, 1989; Hatta *et al.*, 1991; Rossiter *et al.*, 2003). It was the aim of this study to determine whether an increased aerobic capability was borne out at sub-maximal work rates by an extended aerobic range as determined using the v-slope method, (Beaver *et al.*, 1986) and at maximal levels by an increase in aerobic capacity ($\dot{V}O_{2max}$).

3.1 Methods

3.1.1 Subjects

8 men between the ages of 21 and 35 took part in the protocol. All subjects met the standard inclusion criteria (section 2.1 general methods). Table 2 shows the subject characteristics for each individual.

Table 2 - Subject characteristics

Subject	Height (cm)	Body Mass (kg)	Age (yrs)
1	179	61	22
2	182	95	33
3	184	87	35
4	167	71	28
5	170	83	25
6	181	67	21
7	179	81	22
8	186	86	22
Mean	179	79	26
SD	7	11	5

3.1.2 Protocol

All subjects completed a standard incremental protocol until exhaustion on two occasions. The protocol is illustrated in fig. 26. Prior to this, subjects were familiarised with all equipment and procedures on multiple visits to the laboratory. At least one of these visits included an incremental test to exhaustion. DCA or a placebo was administered in a randomised fashion on one of the two visits. All exercise started 1 hour after DCA or placebo administration (see section 2.2.5). The test began with 3 minutes of resting breathing and then 5 min of low load pedalling at 20 W. The work

rate was increased by 15w · min^{-1} (at a rate of 3w · $12s^{-1}$) until voluntary exhaustion or until a cadence of 50 rpm could no longer be maintained. Where possible a 10 min period of recovery data was collected post exhaustion with subjects sat stationary on the ergometer.

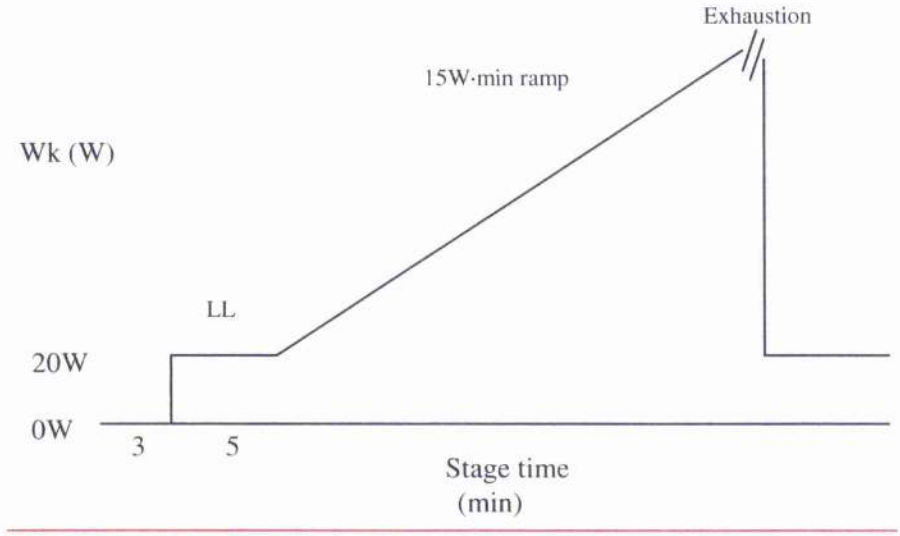


Figure 26 - Schematic representation of the incremental exercise protocol. LL = Low load pedalling.

Blood samples were taken prior to DCA/placebo administration, 4 min into low load pedalling, every 3 min during the ramp, at exhaustion and 3 min, 6 min and 9 min post exhaustion. Ratings of perceived exertion and dyspnoea were taken 45 s before each blood sample and as close to the point of exhaustion as possible.

The techniques for heart rate, gas exchange, and NIRS measurement are described in the general methods (chapter 2).

3.1.3 Analysis

Peak $\dot{V}O_2$ was taken from a moving average of the last 20 s of the ramp data. Peak values were recorded for maximum work rate and maximum heart rate. The lactate threshold was determined by a standard visual identification technique based on the v-slope method (Beaver *et al.*, 1986). Independent checking of the results was undertaken by 2 experienced personnel. Where the results were different an average of the estimates was taken. The threshold is defined as the slope change in a graph of pulmonary $\dot{V}CO_2$ against $\dot{V}O_2$. It is verified by a slope change in plots of E_TO_2 and $\dot{V}E/\dot{V}O_2$ against $\dot{V}O_2$ (Caiozzo *et al.*, 1982; Whipp *et al.*, 1986). The slope of $\Delta\dot{V}O_2/\Delta WK$ was determined by fitting a linear regression to the linear portion of the relationship. Generally, this excluded the first 3 min of data and any evident plateaux in $\dot{V}O_2$ in the last few minutes of the ramp.

3.1.4 Statistics

Statistical analysis followed that described in general methods chapter 2 (section 2.2.10)

Results

3.2 Incremental exercise

3.2.1 Blood borne metabolic responses to incremental exercise

The subject's pre test activity and diet were controlled as described in the general methods. This ensured that they entered each trial in a similar metabolic state. Therefore, as expected, no difference was found in pre dose values for blood [La], pH, standard bicarbonate (SBC) or glucose (Table 3).

Figure 27 illustrates that blood [Lactate] was significantly reduced during unloaded pedalling, and at 3 min, 6 min and 9 min in the DCA trial when compared to the placebo (Table 3). This data is shown more clearly in figure 27(b), which has been re-scaled to shows these time points only. The reduction in blood [lactate] is 39%, 33%, 21% and 15% for each of these time points respectively. As can be seen in figure 27 a reduction in blood [Lactate] was not evident at exhaustion and at time points 3 min, 6 min and 9 min post exercise (Table 3).

There are no significant differences in pH, SBC or glucose at any time (Table 3).

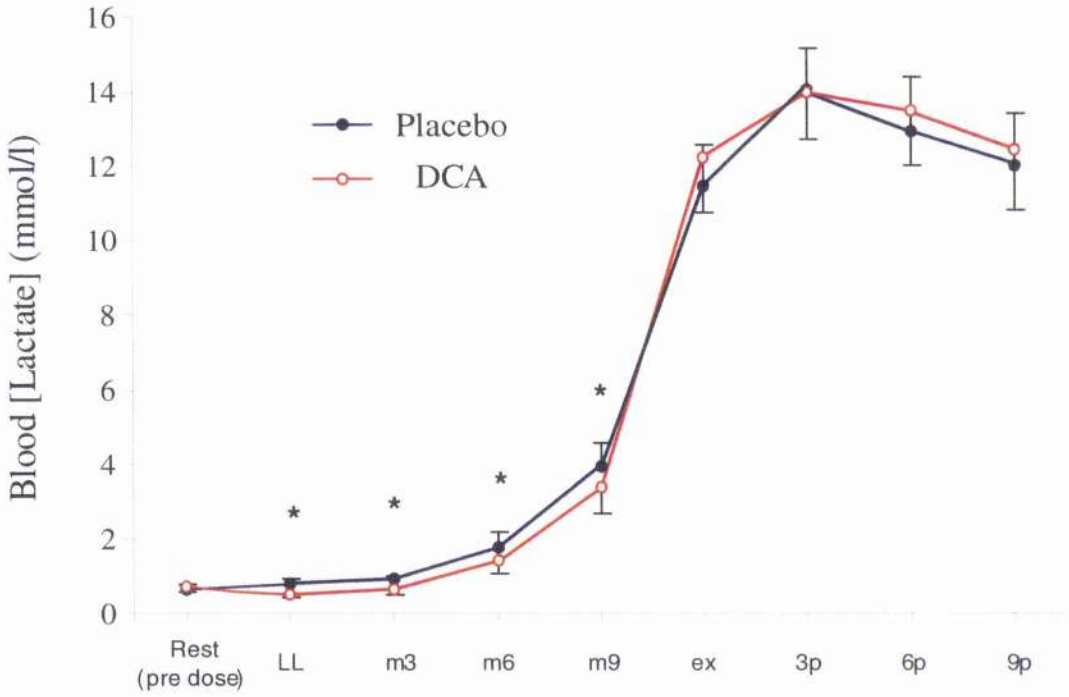


Figure 27 – The effect of DCA on blood [Lactate] during incremental exercise to exhaustion. Data are mean values ± SEM. Closed circles = placebo. Open circles = DCA. Pre dose = Samples taken prior to DCA administration. LL = low load pedalling (20W). m3 = minute 3. m6 = minute 6. m9 = minute 9. ex = exhaustion. 3p = 3 minutes post exhaustion. 6p = 6 minutes post exhaustion. 9p = 9minutes post exhaustion. * Statistically significant between placebo and DCA trials. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at $P<0.05$.

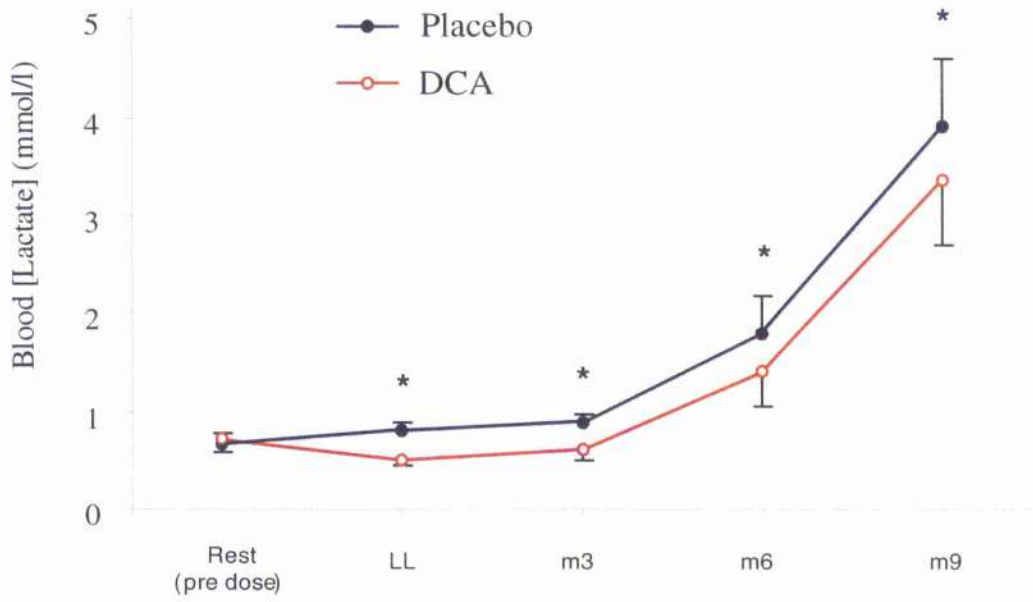


Figure 27b – The effect of DCA on blood [Lactate] during incremental exercise to exhaustion. Figure 27 re-scaled to show more clearly the data points where the difference between trials were significant. Data are mean values + SEM. Closed circles = placebo. Open circles = DCA. Pre dose = Samples taken prior to DCA administration. LL = low load pedalling (20W). m3 = minute 3. m6 = minute 6. m9 = minute 9. * Statistically significant for placebo vs DCA. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired t-test if a main treatment effect was observed. Statistical significance was declared at $P<0.05$.

Table 3 - Blood borne metabolic responses to incremental exercise

	Rest (pre dose)	Baseline (20w)	3 min	6 min	9 min	Exhaustion	3min post	6min post	9min post
La. mmol/l									
Placebo	0.65 ± 0.13	0.80 ± 0.08	0.89 ± 0.09	1.78 ± 0.38	3.92 ± 0.68	11.41 ± 1.12	14.03 ± 1.15	12.90 ± 1.48	12.01 ± 1.38
DCA	0.72 ± 0.15	0.49 ± 0.05*	0.60 ± 0.10*	1.40 ± 0.35*	3.35 ± 0.65*	12.21 ± 1.50	13.99 ± 1.31	13.44 ± 1.45	12.40 ± 1.59
P value	0.32	0.002	0.004	0.002	0.001	0.30	0.95	0.37	0.39
pH									
Placebo	7.41 ± 0.01	7.39 ± 0.01	7.38 ± 0.01	7.37 ± 0.01	7.35 ± 0.02	7.24 ± 0.02	7.20 ± 0.02	7.21 ± 0.02	7.24 ± 0.02
DCA	7.42 ± 0.01	7.40 ± 0.01	7.39 ± 0.01	7.37 ± 0.01	7.36 ± 0.01	7.22 ± 0.02	7.20 ± 0.02	7.21 ± 0.03	7.23 ± 0.03
P value	0.29	0.12	0.13	0.43	0.023	0.15	0.24	0.31	0.18
SBC mmol/l									
Placebo	26.44 ± 0.30	26.15 ± 0.40	26.19 ± 0.39	25.37 ± 0.66	23.70 ± 0.63	16.88 ± 0.91	14.67 ± 0.97	14.95 ± 1.00	15.89 ± 1.03
DCA	26.98 ± 0.33	26.88 ± 0.27	26.75 ± 0.36	25.94 ± 0.63	24.17 ± 0.46	16.26 ± 1.00	14.69 ± 0.94	14.84 ± 1.11	15.76 ± 1.18
P value	0.06	0.16	0.08	0.25	0.26	0.16	0.88	0.76	0.60
Gluc. mmol/l									
Placebo	4.63 ± 0.21	4.92 ± 0.20	4.88 ± 0.19	4.88 ± 0.13	4.84 ± 0.08	4.83 ± 0.14	5.70 ± 0.23	5.62 ± 0.20	5.43 ± 0.21
DCA	4.75 ± 0.14	4.88 ± 0.12	4.78 ± 0.13	4.80 ± 0.12	4.90 ± 0.14	4.91 ± 0.18	5.84 ± 0.15	5.76 ± 0.23	5.62 ± 0.22
P value	0.62	0.85	0.71	0.72	0.77	0.79	0.62	0.59	0.50

Group data (n=8). Data are mean values + SEM. * Statistically significant. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at P<0.05. DCA, dichloroacetate. (pre dose), resting sample taken before DCA administration. DCA = Dichloroacetate; SBC = Standard Bicarbonate; Gluc. = Glucose; La. = Lactate; (pre dose) = pre treatment sample.

3.2.2 $\dot{V}O_2$ response to incremental exercise

Figure 28 shows the gas exchange plots used to estimate the gas exchange threshold (θ_{LT}) for one representative subject (subject 8). Corresponding points on the $\dot{V}CO_2$ vs $\dot{V}O_2$, ETO_2 vs $\dot{V}O_2$ and $\dot{V}E/\dot{V}O_2$ vs $\dot{V}O_2$ plots consistently estimate the lactate threshold (θ_{LT}) at a $\dot{V}O_2$ of 2.25 l/min. 5 of the 8 subjects exhibited an increase in θ_{LT} with DCA administration; however there was no significant difference between groups (Mean $\pm$ SEM. 1.85 $\pm$ 0.14 l/min DCA. 1.78 $\pm$ 0.15 l/min placebo. P = 0.54. Table 4).

No significant difference was observed in $\dot{V}O_2$ during low load pedalling (Mean $\pm$ SEM. 0.76 $\pm$ 0.04 l/min DCA. 0.76 $\pm$ 0.03 l/min placebo. P = 1.0. Table 4). 6 of the 8 subjects had a higher $\dot{V}O_2$ peak post DCA administration; taken as a group mean this equated to a 0.09 L increase (Mean $\pm$ SEM. 3.65 $\pm$ 0.14 l/min DCA. 3.56 $\pm$ 0.14 l/min placebo. Table 4) and this difference proved statistically significant (P= 0.018). There was no significant difference in peak work rate and time to exhaustion between DCA and placebo trials (P = 0.085 and P = 0.26 respectively. Table 4). The $\Delta\dot{V}O_2/\Delta WR$ was 10.1 $\pm$ 0.3 $ml \cdot min^{-1} \cdot W^{-1}$ for the DCA trail and 10.0 $\pm$ 0.5 $ml \cdot min^{-1} \cdot W^{-1}$ for the placebo trial (P = 0.49. Table 4). These values compare favourably with the commonly reported value of 10 $ml \cdot min^{-1} \cdot W^{-1}$ for this measure (see Whipp *et al*, 1982).

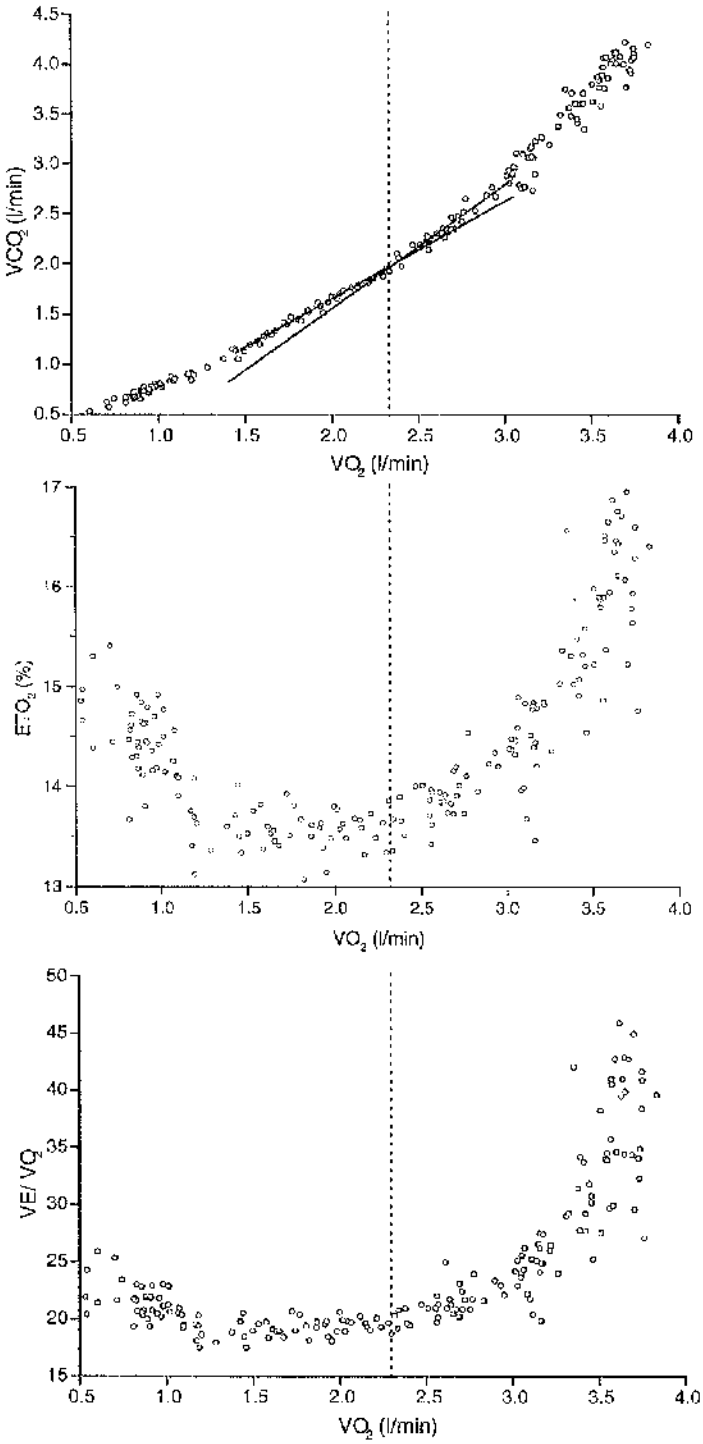


Figure 28 – Gas exchange plots used for the estimation of the lactate threshold. A single representative subject (Subject 8, DCA trial). Top panel: Solid intersecting lines illustrate estimation of lactate threshold using the V – slope method (see section 3.1.3, for a description of this method). The dashed line on all panels represents θ_{LT} at 2.25 (l/min).

Table 4 - $\dot{V}O_2$ response to incremental exercise.

Subject	$\dot{V}O_{2\ 20}$ (l/min)		$\dot{V}O_{2\ peak}$ (l/min)		$\dot{V}O_{2\ LT}$ (l/min)		WR_{peak} (watts)		T_{Lim} (min)		$\Delta\dot{V}O_2 / \Delta Wk$ ($ml \cdot min^{-1} \cdot w^{-1}$)	
	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA
1	0.69	0.68	2.73	2.71	1.05	1.23	240	240	14.75	14.67	9.7	10.2
2	0.89	0.82	4.00	4.00	1.80	2.10	358	368	22.88	23.12	10.1	9.8
3	0.79	0.89	3.59	3.69	2.48	2.39	327	324	20.33	20.10	9.8	9.9
4	0.77	0.70	3.44	3.58	1.89	1.55	312	315	19.40	19.47	10.1	9.8
5	0.90	0.87	3.47	3.71	1.50	1.80	302	317	18.75	19.68	10.7	10.4
6	0.60	0.73	3.70	3.83	1.50	1.90	353	353	22.05	22.15	9.5	10.5
7	0.69	0.64	3.74	3.77	2.02	1.59	386	393	24.37	24.68	9.4	9.9
8	-	0.75	3.78	3.87	2.00	2.25	350	352	22.00	21.90	10.1	10.0
Mean	0.76	0.76	3.56	3.65*	1.78	1.85	325	333	20.57	20.72	10.0	10.1
SEM	0.04	0.03	0.14	0.14	0.15	0.14	16	16	0.04	0.05	0.18	0.09
P value		1.00		0.018		0.55		0.085		0.27		0.49

Data are individual values. Group mean ± SEM data shown. * Statistically significant. Statistical analysis was carried out using a paired *t*-test. Statistical significance was declared at $P<0.05$. 20 = Low load pedalling (20W). $\dot{V}O_2$ peak = average oxygen uptake during the last 20 s of exercise. LT = estimated lactate threshold. T_{Lim} = time to exhaustion. $\Delta\dot{V}O_2 / \Delta Wk$ = delta change in $\dot{V}O_2$ with delta change in work rate taken for the linear portion of the $\dot{V}O_2$ response i.e. excluding the first 3 minutes and the last 2 minutes. Data not available (-).

3.2.2 Cardiovascular responses to incremental exercise.

After administration of DCA the resting heart rate was elevated by as little as 1 beat·min^{-1} (subjects 3 & 5) and by as much as 15 beat·min^{-1} (subject 1), compared to placebo administration. These differences proved statistically significant. (Mean ± SEM. 69 ± 5 beat·min^{-1} DCA. 63 ± 3 beat·min^{-1} placebo. P = 0.033. Table 5). The heart rate continued to be significantly elevated during low load pedalling by an average of 4 beat·min^{-1} (Mean ± SEM. 84 ± 6 beat·min^{-1} DCA. 80 ± 5 beat·min^{-1} placebo. P = 0.0002. Table 5). No significant difference was found in peak heart rate (Mean ± SEM. 185 ± 11 beat·min^{-1} DCA. 184 ± 5 beat·min^{-1} placebo. P = 0.14. Table 5).

The results clearly show an increase in heart rate at rest and during unloaded pedalling during the DCA trial when compared to the placebo. These changes are previously un-described and indicate a possible chronotropic response associated with dichloroacetate administration. This has possible implications regarding the general assumption that DCA has no effect on bulk oxygen delivery during exercise. See section 3.3 for further discussion.

No significant difference was found in O_2 pulse during low load pedalling (Mean ± SEM. 9.0 ± 1.2 ml/beat DCA. 9.5 ± 1.4 ml/beat placebo. Table 5) or between peak exercise values (Mean ± SEM. 19.8 ± 2.9 ml/beat DCA. 19.4 ± 2.9 ml/beat placebo).

Table 5 - Cardiovascular responses to incremental exercise.

Subject	HR_{rest} (bpm)		HR_{20} (bpm)		HR_{peak} (bpm)		O_2Pulse_{20} (ml/beat)		O_2Pulse_{peak} (ml/beat)	
	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA
1	61	76	82	85	190	194	8.4	8.0	14.3	14.0
2	64	70	76	79	161	163	11.7	10.4	24.8	24.5
3	66	67	78	82	174	175	10.1	10.9	20.6	21.1
4	-	-	79	83	186	189	9.7	8.4	18.5	18.9
5	66	67	90	97	190	192	10.0	8.9	18.3	19.3
6	62	75	83	88	194	191	7.2	8.2	19.1	20.1
7	60	66	76	80	185	186	9.0	7.9	20.2	20.3
8	59	63	75	79	192	192	-	9.5	19.7	20.2
Mean	63	69*	80	84*	184	185	9.5	9.0	19.4	19.8
SD	3	5	5	6	11	11	1.4	1.2	2.9	2.9
P value		0.033		0.0002		0.140		0.245		0.100

Data are individual values. Group mean ± SEM data shown. * Statistically significant. Statistical analysis was carried out using a paired *t*-test. Statistical significance was declared at $P<0.05$. HR = heart rate. O_2Pulse = oxygen pulse ($\dot{V}O_2$ /heart rate). Peak = an average of the last 20s of exercise. 20 = low load pedalling (20W). Data not available (-).

As described, heart rate was elevated at rest and during unloaded pedalling for all subjects during the DCA trial. The same consistency of response is not replicated during the ramp exercise.

The group mean ± SEM heart rate responses are shown in figure 29. The clear elevation in baseline heart rate during the DCA trial is not evident early into the incremental protocol. There is a tendency for the heart rate to be higher on the DCA trial but not significantly. When the heart rate values are normalised to baseline values (figure 30), the heart rate responses can be seen to overlay almost exactly.

The group mean ± SEM oxygen pulse responses are shown in figure 30. No significant differences are evident at any time during exercise.

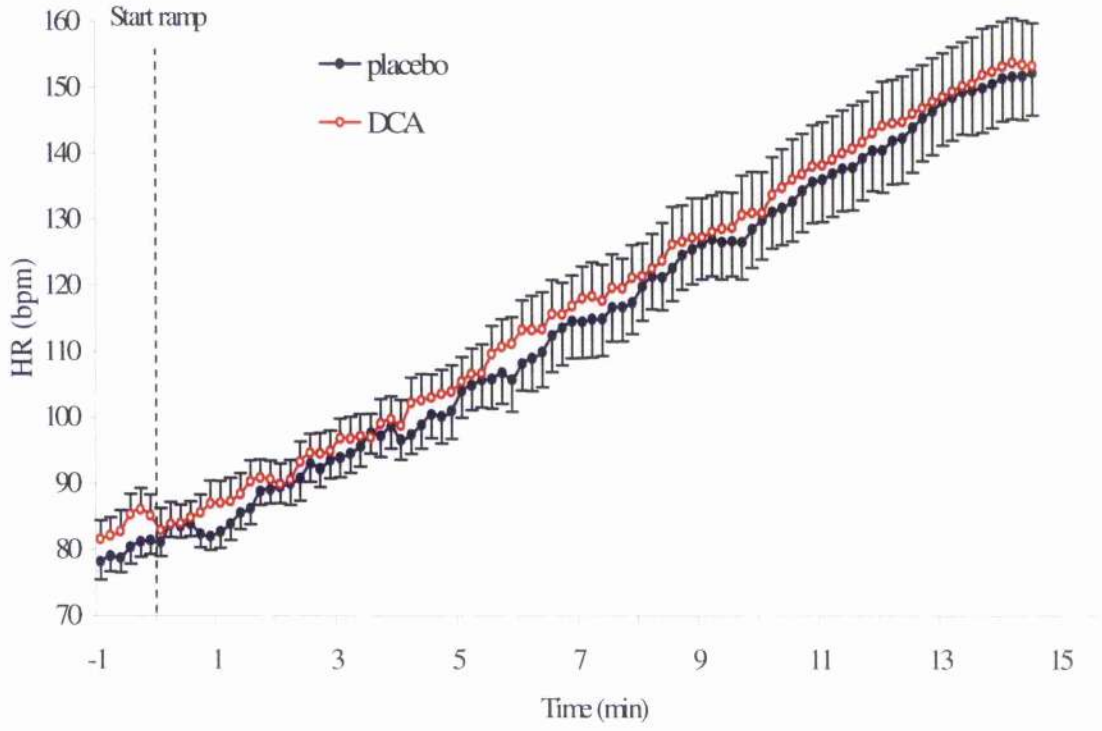


Figure 29 – Heart rate response to incremental exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the ramp increase in work rate from a 20W baseline.

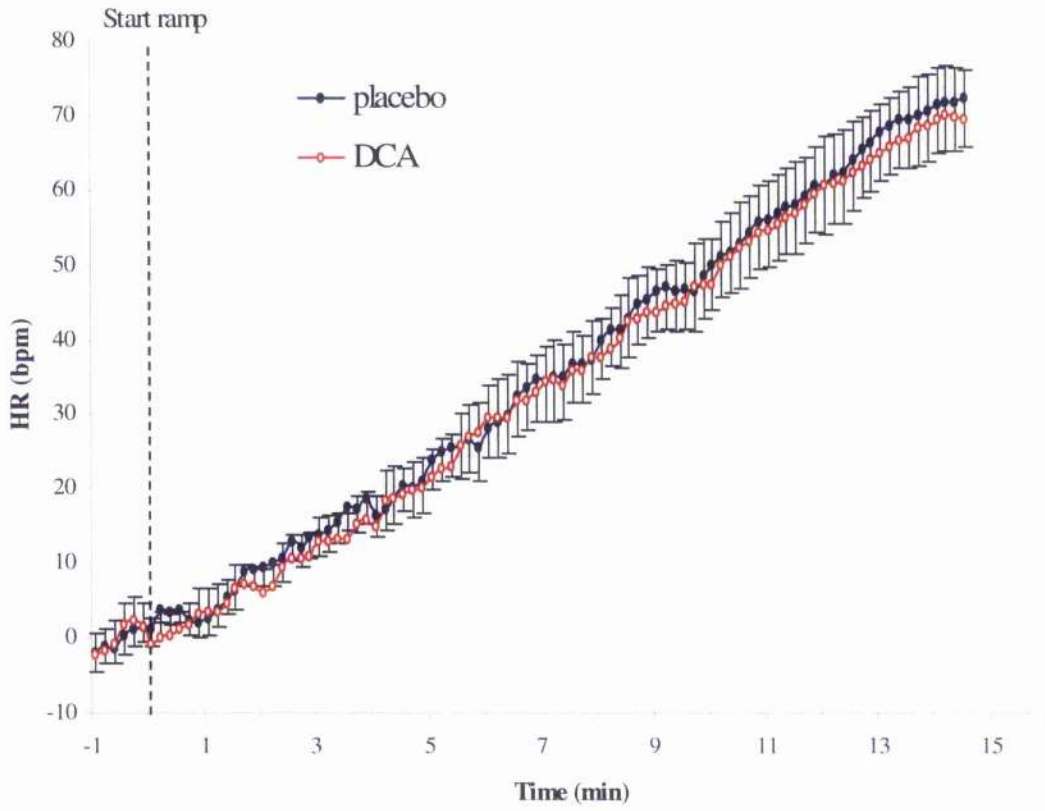


Figure 30 – Heart rate response to incremental exercise normalised to baseline values. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the ramp increase in work rate from a 20W baseline.

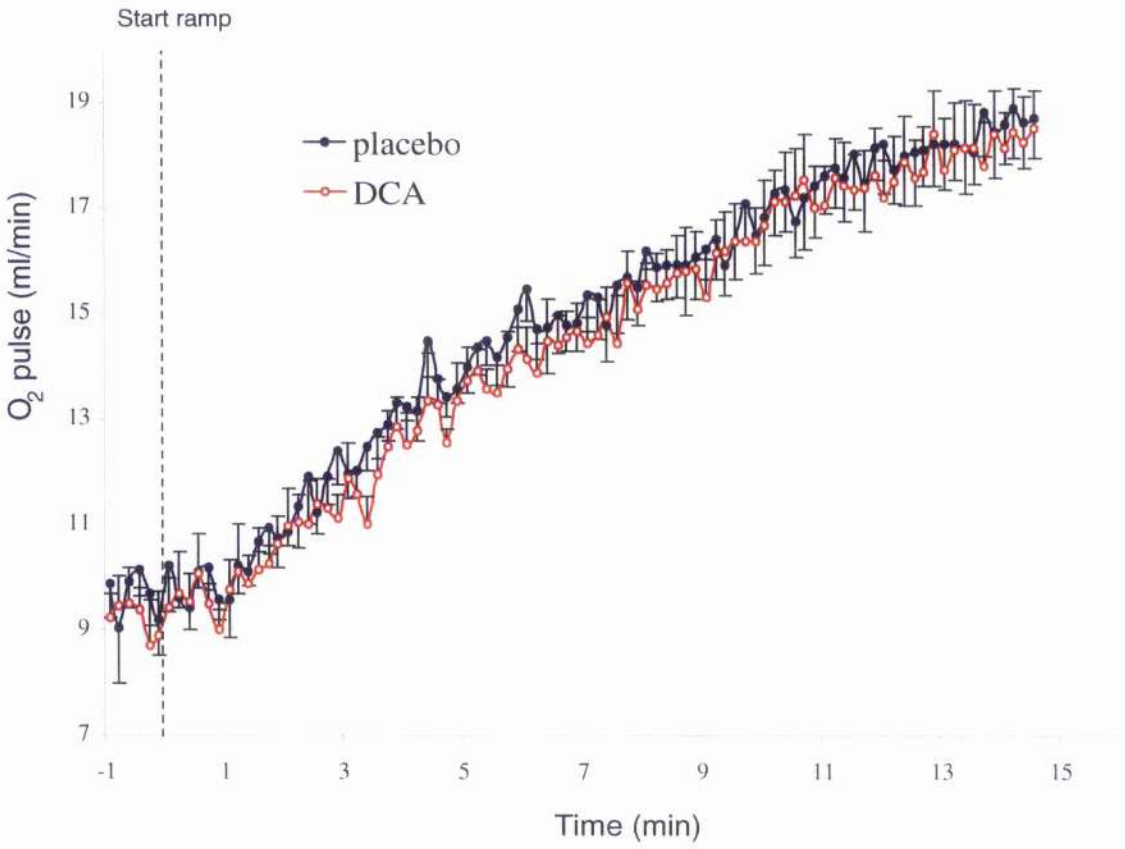


Figure 31 – Oxygen pulse response to incremental exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the ramp increase in work rate from a 20W baseline.

3.2.4 Muscle oxygen saturation responses to incremental exercise

Figure 32 shows the group mean ± SEM delta deoxyhaemoglobin plots. The data is corrected to % maximal MVC and normalised to unloaded baseline. Values for the placebo trial are lower during the first minute of exercise. Thereafter, values differ little between the two trials.

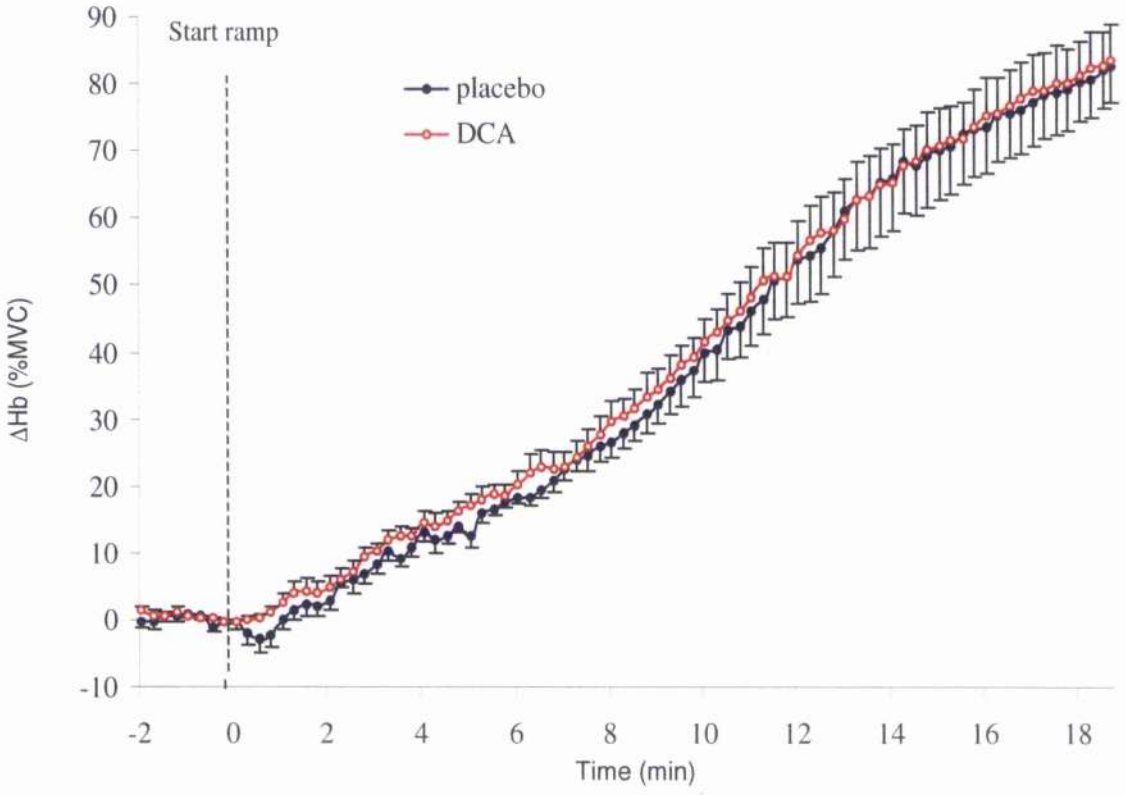


Figure 32 - Delta deoxyhaemoglobin responses to incremental exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the ramp increase in work rate from a 20 W baseline.

3.2.5 RPE and Dyspnoea ratings during incremental exercise.

Figure 33 shows the group means $\pm$ SEM values for rating of perceived exertion. Values are given for rest, the incremental protocol and recovery. Graphically it appears on average the subjects rate exercise easier at 6 out of the 7 sub-maximal time points. However, these differences are not statistically significant. Ratings of dyspnoea (figure 34) followed a similar pattern, however again no significant differences were found.

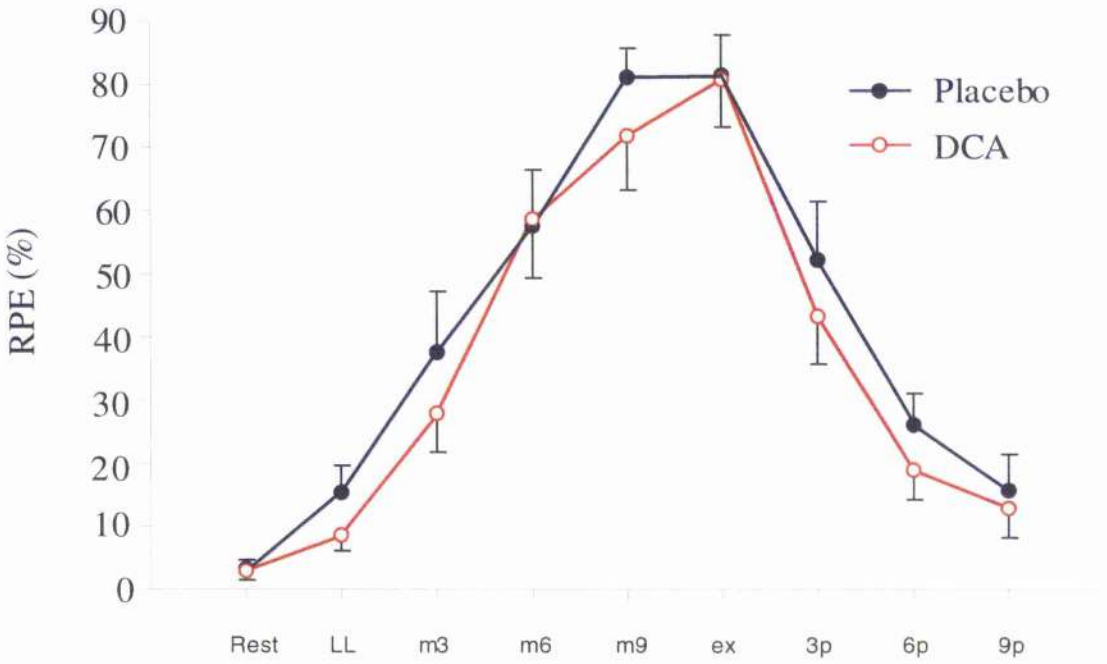


Figure 33 - Ratings of perceived exertion during incremental exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. LL = low load pedalling (20W). m3, m6, m9 = minutes 3, 6, & 9 respectively. Ex = exhaustion. 3p, 6p, 9p = minutes 3, 6, & 9 post, respectively.

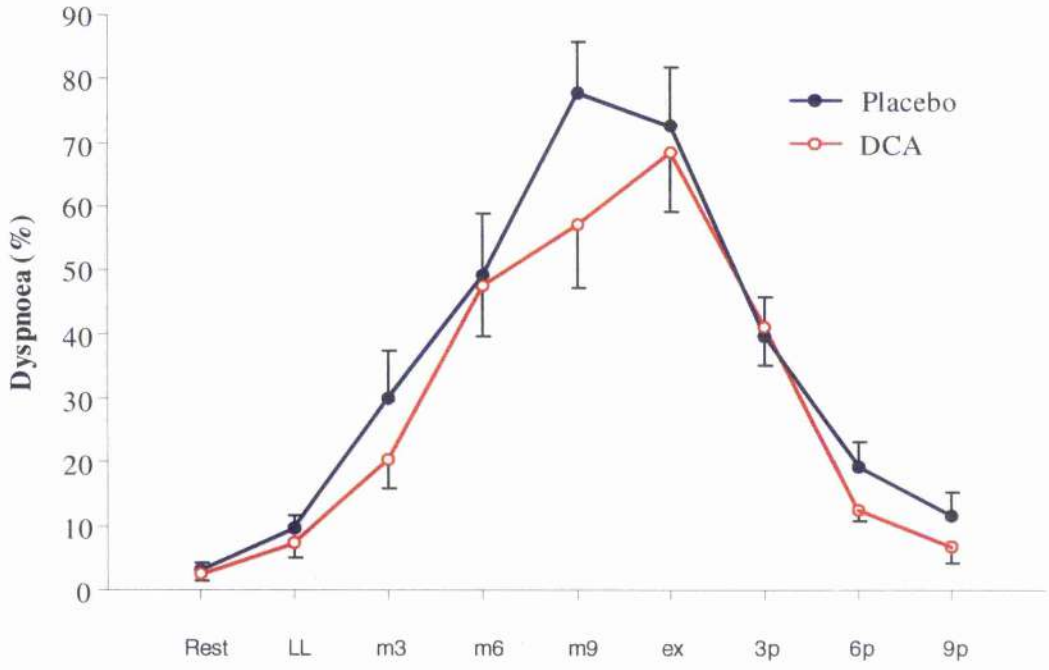


Figure 34 - Ratings of dyspnoea during incremental exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. LL = low load pedalling (20W). m3, m6, m9 = minutes 3, 6, & 9 respectively. Ex = exhaustion. 3p, 6p, 9p = minutes 3, 6, & 9 post, respectively.

Chapter 3

3.3 Discussion

3.3.1 The effect of DCA on $\dot{V}O_2$ max

'Maximal' or 'peak' $\dot{V}O_2$, its description, and the factors determining it have been a subject of debate for the best part of a century (Honig *et al.*, 1992; Wagner, 1992; Green and Palta, 1992; Bassett and Howley, 2000; Noakes, 1997; Bergh *et al.*, 2000; Wagner, 1996; Di Prampero, 1985; Sutton *et al.*, 1988; Saltin *et al.*, 1976; Dempsey *et al.*, 1984). From this debate two principal paradigms have developed: the first advocating oxygen delivery from atmospheric air to the mitochondria of the working muscle as the process setting the upper limit for oxygen uptake, the second suggesting that the demand set by the metabolic machinery within the working muscle is responsible i.e. $\dot{V}O_{2\max}$ is metabolically limited. The principal metabolic effect of DCA, up regulation of pyruvate dehydrogenase, does not easily fit into either of these theories. Therefore the small but significant improvement in $\dot{V}O_2$ max shown in this study is perhaps unexpected.

An initial hypothesis for this study was that the lactate lowering properties of DCA may delay the onset of fatigue and improve exercise performance; and that this may result in an increase in $\dot{V}O_{2\max}$. There was little evidence to support this in this study. Time to exhaustion and maximum work rate were not significantly different between DCA and Placebo trials. Similarly the lactate concentration associated with exhaustion was not significantly different between the two trials. Therefore, the small but significant

increase in $\dot{V}O_{2max}$ observed in this study is unlikely to reflect an increased tolerance to maximal exercise through DCA administration.

These findings are supported by the results reported by Putman *et al* (1993). These investigators suggested that a partial reduction in any 'metabolic limitation' as a result of DCA administration would be unlikely during maximal exercise. They demonstrated that activation of PDH above placebo levels was only evident for 3 min during heavy intensity exercise. Therefore a disparity between PDH activation during the final stages of an incremental protocol to exhaustion is unlikely. The equivalence between peak blood lactate values in this study supports this.

Therefore, if the observed increase in $\dot{V}O_{2max}$ is not a result of a metabolic limitation is it a result of enhanced oxygen delivery? Factors governing the delivery of oxygen from environmental air to the mitochondria of the working muscle include 1) arterial O_2 saturation (reflecting, ventilation, VA/Q inequality in the lung, pulmonary diffusion limitation, and Hb-O_2 affinity); 2) arterial O_2 concentration – that is, O_2 saturation and [Hb]; 3) cardiac output or muscle blood flow; and 4) muscle O_2 extraction (Wagner, 2000). Of these, the only 'evidence' we have of DCA affecting any of these processes is a suggestion that maximal heart rate may be increased. While there was no significant difference between DCA and placebo trials when the groups were taken as a whole, 6 out of the 8 subjects showed an elevated peak heart rate on DCA with one unchanged and one 3 beats higher on the placebo trial (subject 6). With subject 6 removed from the analysis the difference between DCA and Placebo trials becomes highly significant ($P = 0.0107$). While there is no reasonable justification for doing this, it is worth discussing the $\dot{V}O_2$ max changes with a view that an increase in maximum heart rate may have become significant with greater subject numbers. The obvious

inference to make is that an increase in maximum heart rate will augment oxygen delivery by increasing cardiac output, assuming that stroke volume is maintained. The demonstration that elite level athletes may have maximal cardiac outputs of up to twice that of 'ordinary' individuals has long identified it as a key factor influencing maximal oxygen uptake (Blomqvist *et al.*, 1983). Indeed Cerretelli and Di Prampero (1987) estimated that 70 - 85 % of the limitation in $\dot{V}O_2$ max is linked to cardiac output. The influence of cardiac output on oxygen delivery can be illustrated by:

$$totalO_2transport = 1.39 \times [Hb] \times SaO_2 \times \dot{Q}T$$

Eqn 18.

Where, Hb is haemoglobin concentration, SaO_2 is arterial oxygen saturation and $\dot{Q}T$ is pulmonary blood flow/cardiac output (Wagner, 1996).

Cardiac output is clearly a possible determinant of $\dot{V}O_{2max}$. It is changes in stroke volume rather than changes in maximal heart rate that usually determine its influence. For example observed increases in $\dot{V}O_{2max}$ as a result of training have been attributed to increasing stoke volume rather than changes in maximum heart rate (Saltin *et al.*, 1968; Ekblom *et al.*, 1968).

There is no means in this study to support any increase in maximum cardiac output due to an increase in stroke volume. The effects of DCA on haemodynamics are not well documented (see section 6.8 for further discussion). In relation to this study however it is worth noting that Stacpoole *et al* (1983) reported an increased cardiac output in critically ill patients with hypotension and lactic acidosis. These findings are supported by Ludvik *et al* (1991) who reported an increased cardiac output and enhanced oxygen

delivery as result of DCA administration in healthy volunteers. This increase in cardiac output was independent of any change in heart rate and therefore related to an augmented stroke volume. In this study we have demonstrated an increase in heart rate at rest, unloaded pedalling and suggested that with a greater number of subjects that this may have also been evident at maximal levels. The possible reasons for this increase in heart rate and its disparity with previous studies will be discussed further in the general discussion (Section 6.8). However, on the basis of the studies of Ludvik *et al* (1991) and Stacpoole *et al* (1983), a positive inotropic effect of DCA on stroke volume at maximal work rates in this study cannot be ruled out. Therefore a haemodynamically mediated improvement in $\dot{V}O_{2max}$ as a result of DCA administration is not beyond reason and warrants further investigation.

It is worth noting that increases in $\dot{V}O_{2max}$ did not always go hand in hand with changes in maximum heart rate. For instance subject 6 whose heart rate was 3 beats lower on DCA showed a 0.13 l/min increase in $\dot{V}O_{2max}$ and subject 1 who showed a 4 beat increase in heart rate for the DCA trial showed essentially no difference in $\dot{V}O_{2max}$.

$\dot{V}O_2$ max in athletic populations is thought to be more limited by pulmonary O_2-supply, while non-athletic populations are thought not to be pulmonary O_2-supply limited (Karas *et al.*, 1987). This was demonstrated by Karas *et al* (1987) who showed an increase and decrease in $\dot{V}O_2$ max in athletic subjects with increasing and decreasing F_IO_2. In untrained subjects they reported no increase in $\dot{V}O_2$ max with increasing F_IO_2, nor a significant loss in $\dot{V}O_2$ max as a result of moderate reductions in F_IO_2. While the

subjects in this study trained regularly and were well familiarised with cycling, the highest $\dot{V}O_{2max}$ of 55 ml·min·kg^{-1} (mean ± SD 45±5 ml·min·kg^{-1}) is below that expected for a well trained endurance athlete (>65 ml·min·kg^{-1}). Therefore mechanisms other than an augmented O_2 delivery are perhaps more likely to show improvements in $\dot{V}O_{2max}$ in this subject group.

DCA activates pyruvate dehydrogenase in nearly all mammalian tissues, the testes and gut seemingly the only exceptions (Stacpoole, 1989). Therefore an increase in pyruvate flux into the mitochondria and subsequent greater metabolism of lactate in tissues such as liver cells may increase oxygen consumption above that required for force production. Whipp (1994) calculated the additional O_2 cost of lactate oxidation in the liver during exercise to be approximately 17 ml/min. This was based on a blood lactate of 7 mmol/l, a liver blood flow of 0.05 l/min and a 50% extraction. Making an assumption that the ~40% reduction in blood lactate seen during unloaded pedalling reflects a proportionate increase in liver lactate oxidation only, this would increase the 17 ml/min estimate of Whipp's. Therefore, some of the increase in $\dot{V}O_{2max}$ seen in this study may be apportioned to an up regulation of lactate oxidation in liver and/or other tissues not directly involved in force production. A further contribution from skeletal muscle to lactate metabolism may also be significant in the augmented $\dot{V}O_{2max}$. Whipp (1994) estimated the value for lactate oxidation through this mechanism could theoretically be as high as 660 ml/min. However, he argues that contracting muscle units are unlikely to be undergoing glycogenesis from lactate on thermodynamic grounds, whereas the non lactate generating units are unlikely to be sufficiently glycogen depleted to constitute an available terminus for the resynthesis. However, it may be possible that DCA up regulates this process in non-contracting units subsequently increasing O_2 consumption. The results from this study show that lactate

was significantly reduced throughout exercise. Therefore, a significant increase in $\dot{V}O_2$ due to increased lactate oxidation should also have been evident at rest and during the early stages of exercise. It is obvious from the $\dot{V}O_2$ results that this is not the case.

$\dot{V}O_2$ was statistically different between the 2 trials at the end of exercise when blood lactate concentrations are not significantly different. An increased peak $\dot{V}O_2$ due to increased lactate oxidation by non-contracting muscle units is plausible and this mechanism may make a small contribution to the evident increase in $\dot{V}O_{2max}$. However, the results from submaximal exercise would suggest this is not the case.

Impaired pulmonary function may also prove a limitation to $\dot{V}O_{2max}$. High rates of blood and gas flow could result in their non-uniform distribution within the lungs. This would cause ventilation/perfusion (V_A/Q) mismatch and reduce arterial PO_2 as a result. Furthermore because $\dot{V}O_2$ rises relatively more than cardiac output, mixed venous PO_2 falls. This could be to the extent that if there are any intra-pulmonary or post-pulmonary shunts, arterial PO_2 will be reduced more during exercise than at rest due to the lower PO_2 of the blood (venous) that is carried. The net effect is an increase in the alveolar-arterial PO_2 difference to ~30 mmHg at maximal exercise compared to 5-10 mmHg at rest (Wagner, 1996). A major contribution to this increase is VA/Q inequality (Gledhill *et al.*, 1982). Ludvik *et al* (1991), Stacpoole *et al* (1983) and Wargovich *et al* (1988) reported decreases in peripheral vascular resistance resulting from dichloroacetate administration. Therefore a similar effect on the lung vasculature, perhaps coupled with an increased cardiac output/ pulmonary blood flow may result in a reduction of this VA/Q inequality. While this cannot be ruled out as a mechanism for increasing $\dot{V}O_{2max}$ in this subject group, a pulmonary limitation to $\dot{V}O_{2max}$ is again

thought to be reserved for more athletic subjects (in healthy subject groups). This relates again to studies showing the invariability of $\dot{V}O_{2max}$ in untrained subjects with altering F_IO_2 and no evident desaturation of arterial PO_2 during maximal exercise (Lawler *et al*, 1988; Roca *et al*, 1992).

While the increase in $\dot{V}O_{2max}$ in this study was small (0.09 l/min) it remains statistically significant. However, a small but improved oxygen uptake at maximal exercise intensities was not reflected in an increase in maximal work rate, time to exhaustion or a reduction in peak lactate. Therefore in terms of an improved ability to perform exercise it is not proven to be important. Making an assumption that the delta $\dot{V}O_2$ / delta work rate relationship of 10.1 ml·min·W^{-1}, calculated from the incremental responses in this study, holds for an increase in either variable at these work intensities. The 0.09 l/min increase in $\dot{V}O_{2\,max}$ would equate to an additional ~ 9 watts. While a significant increase in work rate is not demonstrated in this study, an increase of this magnitude in an athletic population would be significant in events where $\dot{V}O_{2\,max}$ is considered to be limiting to performance.

Roberts *et al* (1997) is the only other study to look at the effects of dichloroacetate on incremental exercise performance. Using the same dosing procedure as this study they showed no difference in $\dot{V}O_{2\,max}$ or time to exhaustion (n= 7). However lactate was lowered at rest, between 80-100% of $\dot{V}O_{2\,max}$ and throughout 8 min of recovery. While their findings contradict those found in this study it is difficult to make detailed comparisons because the Roberts *et al* (1997) study was only published in abstract form.

On the basis of these findings further investigation of the effect of DCA on maximal exercise performance is warranted, with an emphasis on a better description of the athletic ability of the population group and closer look at haemodynamic changes.

3.3.2 The effect of DCA administration on the lactate threshold.

The proposed reduction in oxygen deficit (Timmons *et al.*, 1996) and reduction in blood lactate concentration (Merill *et al.*, 1980; Durkot *et al.*, 1995; Schneider *et al.*, 1981; Carraro *et al.*, 1989; Hatta *et al.*, 1991; Rossiter *et al.*, 2003) upon DCA administration suggest that the contracting muscle units are functioning more aerobically during exercise. It was hypothesised for this study that this may be manifest in an increased $\dot{V}O_2$ associated with an increased blood lactate concentration above an initial baseline i.e. a rightward shift in $\dot{V}O_2\theta$ as estimated by the v-slope method described by Beaver *et al* (1986). However, the results reported in this thesis show there was no significant difference in $\dot{V}O_2\theta$ between the DCA and placebo groups. This is somewhat surprising as lactate concentration was significantly reduced throughout sub-maximal exercise for the DCA condition compared to the placebo. This was not accompanied by a significant change in either pH or standard bicarbonate measures. At first glance this seems to be contrary to what may be expected and initially hypothesised at the beginning of this study. However it is clear from Figure 27 that while blood lactate concentration remains lower during sub-maximal exercise, it differs only in absolute term. The increase above an initial baseline occurs at the same time point for both trials i.e. the exercise blood lactate curve in the DCA condition has been displaced downwards rather than rightwards. Therefore while the magnitude of the blood [lactate] response is different between the two conditions θ_{LT} is unlikely to be different. This

would explain why no difference was seen in θ_{LT} for the DCA trial compared to the placebo trial, despite a significant reduction in blood lactate concentration being observed throughout the submaximal exercise range.

Several mechanisms have the potential to increase lactate production as $\dot{V}O_2$ increases during exercise. These include, an increase in glycolytic rate producing pyruvate in quantities greater than that that can be driven through PDH, a reduction in cytosolic redox state ($NADH/NAD^+$) due to the rate of oxidative phoshorylation being unable to sustain the re-oxidation of cytosolic $NADH^+$, increased recruitment of Type IIb muscle fibres and a reduction in capillary PO_2 to a level that impairs oxygen diffusion into the mitochondria. The effect of DCA on lactate production and the mechanism/mechanisms behind this will be discussed in further detail in the general discussion (section 6.9).

3.3.3 Summary of findings

DCA did not improve performance of the incremental exercise protocol for the subject group in this study. This was reflected by no difference in maximum work rate, time to exhaustion or lactate threshold between the DCA and placebo trials. There was however an unexpected, small, but significant increase in $\dot{V}O_{2max}$ for the DCA trial compared to the placebo trial. It is suggested here that this small increase in $\dot{V}O_{2max}$ may reflect haemodynamic changes associated with DCA. However no direct evidence is available from these studies to support this theory. These findings suggest that further investigation of the effect of DCA on maximal exercise performance and associated haemodynamic changes is warranted.

Chapter 4

The effect of Dichloroacetate on oxygen uptake during 'moderate' intensity exercise

Chapter 4

4.0 Introduction

Dichloroacetate has been shown to increase the activity of the Pyruvate dehydrogenase complex (PDH) at rest and during exercise (Stacpoole, 1989; Timmons *et al.*, 1998; Howlett *et al.*, 1999a&b; Gibala and Saltin, 1999; Parolin *et al.*, 2000). A reduction in phosphocreatine degradation subsequent to this has led to the suggestion that a relatively slow activation of PDH could contribute to the lag in oxidative ATP regeneration at the start of exercise. Indeed Timmons *et al* (1998) interpreted these results as evidence that PDH activation played a major role in determining the O_2 deficit at the onset of exercise. However, this relationship was inferred from PCr measurements. There was no direct measurement of $\dot{V}O_2$, neither were there enough data points to allow kinetic discrimination. The aim of this study was to examine the effect of DCA on the fundamental $\dot{V}O_2$ kinetics during moderate intensity exercise. Working within the moderate exercise intensity domain eliminates the complicating influence of the $\dot{V}O_2$ slow component. This enables a more accurate modelling of the fundamental $\dot{V}O_2$ response thereby ensuring a more accurate reflection of $\dot{Q}musO_2$ (Barstow *et al.*, 1990; Grassi *et al.*, 1996).

Chapter 4

4.1 Methods

4.1.1 Subjects

6 male subjects between the ages of 21 and 35 took part in the protocol. All subjects met the standard inclusion criteria (section 2.1 general methods). Table 6 shows the subject characteristics for each individual.

Table 6 - Subject characteristics

Subject	Height (cm)	Body mass (kg)	Age (yrs)
1	179	61	22
2	182	95	33
3	184	87	35
4	167	71	28
5	170	83	25
6	186	86	22
Mean	178	80	28
SD	8	12	6

4.1.2 Protocol

Prior to undertaking this study subjects were familiarised with all equipment and procedures on multiple visits to the laboratory. Prior to completing the DCA/Placebo trials subjects underwent an incremental exercise test to exhaustion for the determination of $\theta_{L/T}$, WR_{max} and $\dot{V}O_{2max}$. On two subsequent visits subjects completed a double, sub-threshold, square wave protocol (fig. 36) consisting of: rest (4 min), low load pedalling (20 W, 5 min), exercise bout 1 (7 min), low load pedalling (20 W, 7 min), exercise bout 2 (7 min), low load pedalling (20 W, 7 min).

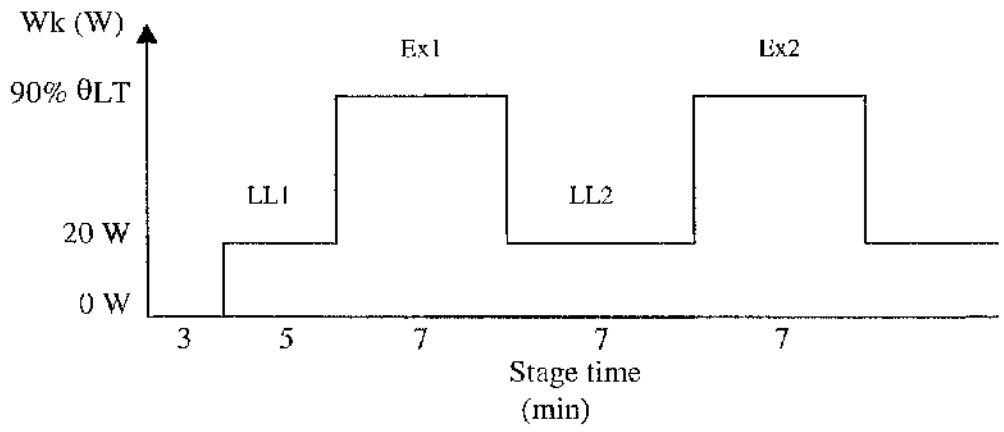


Figure 35 - Schematic illustrating the Sub threshold exercise protocol . LL1 and LL2, represent the first and second periods of low load pedalling respectively Ex1 and Ex2, represent the first and second periods of loaded pedalling respectively. The exercise workload was set at 90% of each individuals estimated lactate threshold (θ_{LT}). The solid line illustrates the square wave nature of the work rate increase.

The work rate change for each individual was set at 90% of the workload corresponding to the estimated lactate threshold (θ_{LT}), determined from a standard ramp-incremental exercise protocol. The coefficient of variance of the $\dot{V}O_2$ response and the delta work rate (ΔWR) have been shown to be inversely related (*Eqn.* 19). Therefore, this workload provided the maximum change (delta) in work rate, allowing a margin for error, so as not to exceed θ_{LT}.

$$\Delta WR = 1/CV$$

Eqn 19.

DCA administration and blood handling followed the procedures described in section 2.2.5. Blood samples were taken at rest prior to DCA administration, 1 min before the start of each square wave exercise bout and at the end of the 6th min of each square wave. Ratings of perceived exertion and dyspnoea were taken 45 s before each blood sample. Heart rate, gas exchange, and NIRS measurements were made as described in chapter 2.

4.1.3 Analysis

Each $\dot{V}O_2$ data set was edited, as described in the general methods (Chapter 2). Gaussian distribution characteristics were assumed as described by Lamarra *et al* (1987) and Rossiter *et al* (2000). The $\dot{V}O_2$ response for each test was then interpolated on a second-by-second basis. This allowed subsequent superimposition and averaging of the responses from the 2 repeated transitions.

A commercial software package (Microcal Origin) was used to model the phase II $\dot{V}O_2$ response to each square-wave on-transient. A mono-exponential function was fit to the data *(Eqn.* 20). The fit excluded the 'cardiodynamic' or phase I region (i.e. approximately 20 s after exercise onset), and continued through at least one minute of steady state data (Whipp *et al.*, 1982).

$$\dot{V}O_{2t} = \dot{V}O_{20} + \Delta\dot{V}O_{2ss}\left[1 - e^{-(t-\delta)/\tau}\right]$$

Eqn 20.

$\dot{V}O_{20}$ is the value of $\dot{V}O_2$ at t = 0, $\Delta\dot{V}O_{2(SS)}$ is the asymptotic value to which $\dot{V}O_2$ is assumed to project, τ is the time constant of the response and δ is a delay term similar to (but not equal to) the phase I-phase II transition time (Whipp *et al.*, 1882; Whipp and Ozcyener, 1998). Best-fit values for τ and δ were found for each variable by an iterative least-squares process. The 'goodness' of fit was determined by observing the flatness of the associated residuals and minimising the sum squared errors (i.e. χ^2).

The off-transient response was modelled in the same fashion with the polarity of the equation switched from positive to negative.

The fit for the calculation of the oxygen deficit (O_2 def) was constrained to start at the beginning of exercise (Whipp *et al.*, 1982). The generated time constant (τ') represents a mean response and allows the calculation of the oxygen deficit using the following relationship (Whipp *et al.*, 1982):

$$O_2def = \Delta\dot{V}O_{2ss} \cdot \tau' \qquad \textit{Eqn 21.}$$

4.1.4 Statistics

Statistical analysis followed that described in general methods chapter 2 (section 2.2.10)

Chapter 4

4.2 Results

4.2.1 Blood borne metabolic responses to moderate intensity exercise

Figure 36 shows the results of analysis of blood samples taken during these experiments. Blood [lactate] was not significantly different in the control period for both experiments (Mean ± SEM. 0.83 ± 0.14 mmol/l DCA. 0.74 ± 0.16 mmol/l placebo. P = 0.67. Table 7). However, it was significantly reduced throughout the exercise protocol in the DCA trial. A reduction of 50% in the group mean lactate concentration was observed across all four time points. These data are shown in table 7. Importantly, no significant increase in blood lactate concentration was found during exercise. Indicating that exercise was undertaken at work rates below that associated with the lactate threshold. No significant change in pH was observed, there was a tendency for the standard bicarbonate concentration to increase and for the blood glucose concentration to decrease . However, these did not reach statistical significance.

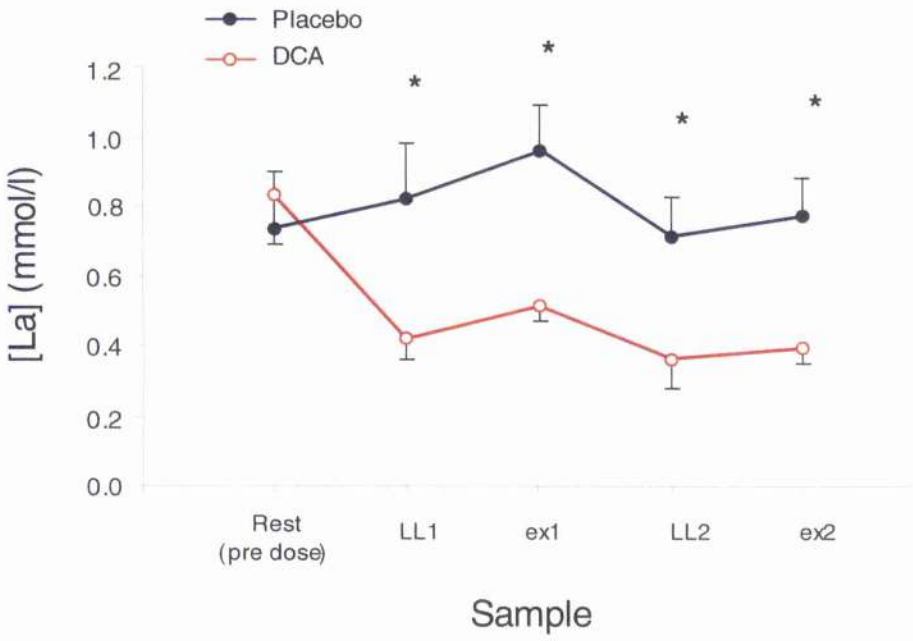


Figure 36 - The effect of DCA on blood lactate during moderate intensity exercise. Data are group means ± SEM. Closed circles = placebo. Open circles = DCA. Data shows that the administration of DCA successfully reduced blood [Lactate] during the sub-threshold exercise protocol, compared to the placebo trial. Furthermore, there was no significant rise in blood [Lactate] during either trial. Indicating that exercise was indeed taking place in the 'moderate' domain. Pre dose = Samples taken prior to DCA administration. LL = low load pedalling (20W). Ex = exercise sample. * Statistically significant between placebo and DCA trials. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at $P<0.05$.

Table 7 - Blood borne metabolic responses to moderate intensity exercise

	Rest (pre dose)	LL 1	Ex 1	LL 2	Ex 2
Lactate mmol/l					
Placebo	0.74 ± 0.16	0.82 ± 0.15	0.96 ± 0.13	0.72 ± 0.11	0.77 ± 0.11
DCA	0.83 ± 0.14	0.42 ± 0.06*	0.52 ± 0.05*	0.36 ± 0.08*	0.39 ± 0.05*
P value	0.67	0.016	0.011	0.001	0.006
pH					
Placebo	7.41 ± 0.005	7.39 ± 0.003	7.37 ± 0.005	7.40 ± 0.006	7.39 ± 0.007
DCA	7.42 ± 0.009	7.39 ± 0.004	7.37 ± 0.002	7.40 ± 0.002	7.39 ± 0.001
P value	0.48	0.91	0.70	0.90	0.45
SBC mmol/l					
Placebo	26.07 ± 0.24	26.14 ± 0.59	25.74 ± 0.28	25.95 ± 0.20	26.04 ± 0.26
DCA	26.38 ± 0.83	26.44 ± 0.70	26.14 ± 0.47	26.41 ± 0.50	26.41 ± 0.56
P value	0.37	0.40	0.072	0.14	0.12
Glucose mmol/l					
Placebo	4.80 ± 0.09	4.83 ± 0.12	4.82 ± 0.14	4.82 ± 0.12	4.83 ± 0.13
DCA	4.76 ± 0.09	4.78 ± 0.11	4.78 ± 0.14	4.72 ± 0.07	4.67 ± 0.09
P value	0.66	0.50	0.30	0.12	0.08

Data are group mean values + SEM (n = 6). * Statistically significant between DCA and placebo trials. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at $P<0.05$. DCA = dichloroacetate. 20 = low load pedalling (20 W) sample. Ex = exercise sample. (pre dose) = resting sample taken before DCA administration. DCA = Dichloroacetate; SBC = Standard Bicarbonate; Gluc. = Glucose; La. = Lactate; (pre dose) = pre treatment sample.

4.2.2 $\dot{V}O_2$ responses to moderate intensity exercise

The group mean ± SEM on-transient $\dot{V}O_2$ responses are shown in figure 37. There are no differences between DCA and placebo trials. The time constant for the conditions (mean ± SEM) was 18.3 ± 1.22 and 16.1 ± 2.02 respectively (Table 8). The time constant for both conditions lie within the normal range previously reported for moderate cycle ergometry exercise (Whipp *et al.*, 1982; Lamarra *et al.*, 1987). The 2 s difference between the mean values is not statistically significant (P = 0.089). Figures 39 a&b show mono-exponential fits for the $\dot{V}O_2$ responses for all subjects. The initial 'cardiodynamic' phase (~25 s) was excluded and the fit continued through at least 1 minute of the steady state. The responses are generally well described by equation 20; that is the associated residuals fluctuate closely and randomly about zero. The residuals for subject 5 (fig. 38b) however show significant portions below zero for the placebo trial. This represented the best estimate of the time course of the response. Therefore, this data has been left in the analysis.

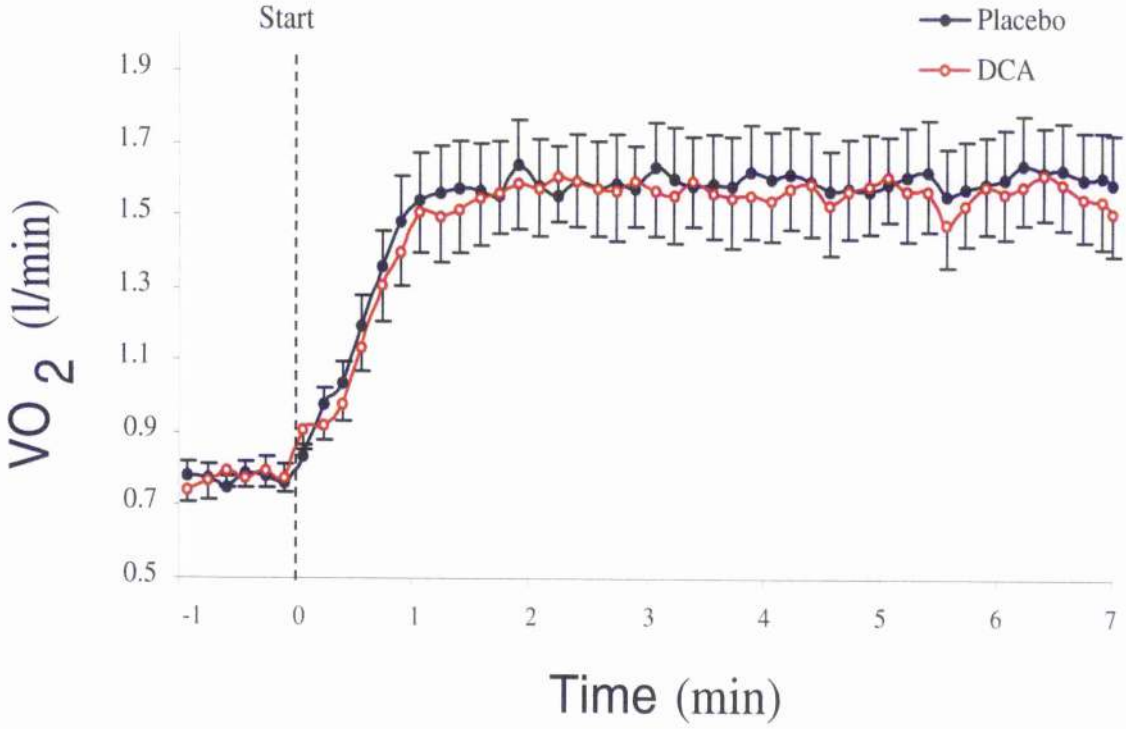


Figure 37 – Data are group mean ± SEM. The on-transient $\dot{V}O_2$ response to moderate square wave exercise. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

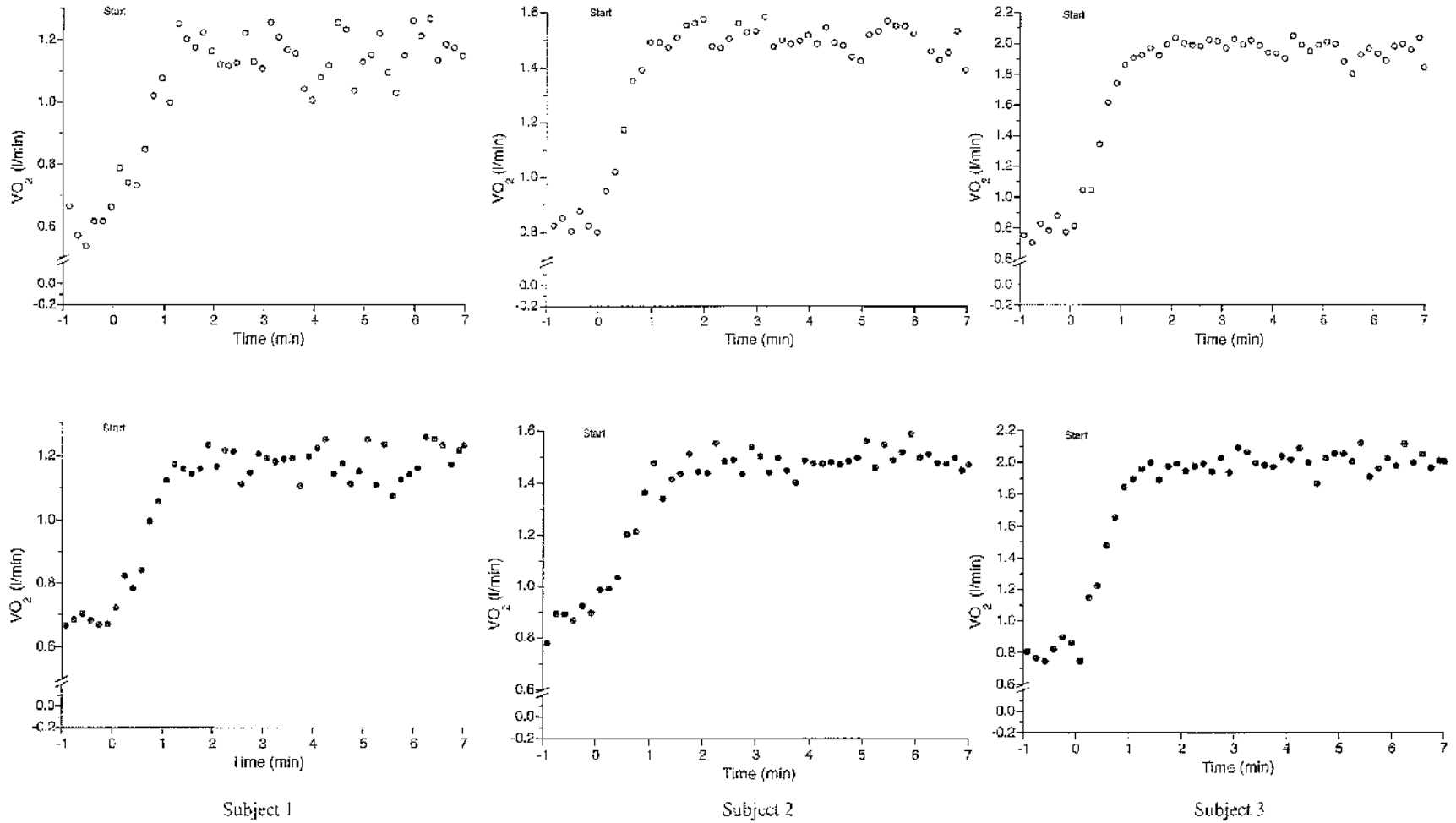


Figure 38a – On- transient $\dot{V}O_2$ response to moderate square wave exercise. Individual plots. Subjects 1-3. Open Circles = DCA (top panels). Closed circles = placebo (bottom panels). The solid line through the data points represents the mono- exponential fit to the data. The fit began at least 20 s into exercise to exclude the cardiodynamically mediated 'phase 1' response and continued through at least 1 min of the steady-state response ('phase 3'). The goodness of fit is illustrated by the fluctuations of the residuals around zero. The dashed line indicates the start of the stepped increase in work rate.

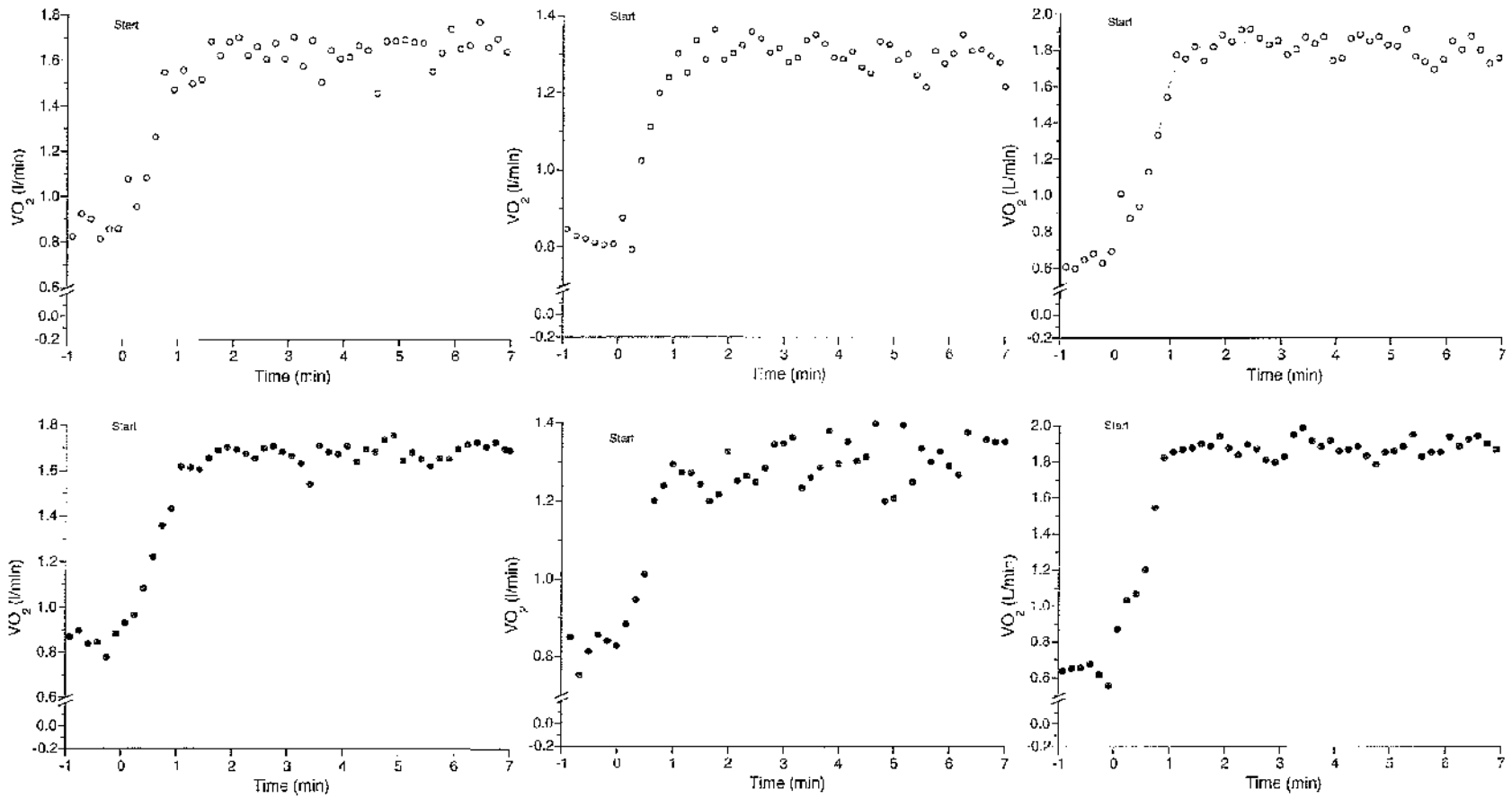


Figure 38b – On- transient $\dot{V}O_2$ response to moderate square wave exercise. Individual plots. Subjects 4-6. Open Circles = DCA (top panels). Closed circles = placebo (bottom panels). The solid line through the data points represents the mono- exponential fit to the data. The fit began at least 20 s into exercise to exclude the cardiodynamically mediated 'phase 1' response and continued through at least 1 min of the steady-state response ('phase 3'). The goodness of fit is illustrated by the fluctuations of the residuals around zero. The dashed line indicates the start of the stepped increase in work rate.

Table 8 - $\dot{V}O_2$ responses to moderate square wave exercise

Subject	on-τ (s)		off-τ (s)		LL $Vo_{2\,ss}$ (l/min)		Ex $Vo_{2\,ss}$ (l/min)		$\Delta Vo_{2\,ss}$ (l/min)		O_2 def (l/min)		$\Delta Vo_{2\,ss}/\Delta$ wk ($ml.min^{-1}.W^{-1}$)	
No.	Plac.	DCA	Plac.	DCA	Plac.	DCA	Plac.	DCA	Plac.	DCA	Plac.	DCA	Plac.	DCA
1	17.3	18.4	29.0	32.3	0.64	0.61	1.18	1.16	0.54	0.55	0.41	0.37	10.4	10.6
2	15.6	16.4	27.2	25.5	0.87	0.88	1.52	1.47	0.65	0.59	0.38	0.45	9.6	8.7
3	21.4	22.7	26.1	35.4	0.80	0.77	1.66	1.58	0.86	0.81	0.82	0.88	7.5	7.1
4	21.4	20.9	25.3	23.8	0.82	0.83	2.02	2.06	1.20	1.23	0.65	0.54	14.6	15.0
5	9.9	16.3	25.5	20.6	0.82	0.82	1.29	1.31	0.47	0.49	0.30	0.33	9.6	10.0
6	11.0	15.0	39.0	31.6	0.65	0.64	1.88	1.84	1.23	1.20	0.72	0.94	10.5	10.3
Mean	16.1	18.3	28.7	28.2	0.77	0.76	1.59	1.57	0.83	0.81	0.55	0.59	10.4	10.3
SEM	2.02	1.22	2.14	2.34	0.04	0.04	0.13	0.14	0.13	0.14	0.09	0.11	0.95	1.08
P value		0.089		0.089		0.32		0.29		0.43		0.44		0.71

Data are individual values. Group mean ± SEM data shown. * Statistically significant. Statistical analysis was carried out using a paired *t*-test. Statistical significance was declared at $P<0.05$. LL = Low load pedalling (20W). Ex = exercise. τ = tau. $\Delta\dot{V}O_{2\,ss}$ = delta steady-state oxygen uptake. O_2 def = oxygen deficit. $\Delta Vo_{2\,ss}/\Delta$ wk = steady state gain in oxygen uptake for an increase in work rate.

The data in table 8 show that the values for the unloaded pedalling baseline, and exercise steady-state, $\dot{V}O_2$ were very similar for all subjects in the group. No significant difference was found between the two conditions. Thus there is no difference in the steady-state $\dot{V}O_2$ gains (Mean ± SEM. 10.3 ± 1.08 ml·min^{-1}·W^{-1} DCA. 10.4 ± 0.95 ml·min^{-1}·W^{-1} placebo. P = 0.71). The values for both conditions are comparable to the value of 10 ml·min^{-1}·W^{-1} reported by Wasserman & Whipp (1975) and Hansen *et al* (1984) and the values reported for the incremental work protocol in chapter 3. As expected from the above results, the calculated O_2 deficit (Mean ± SEM) of 0.59 ± 0.11 l/min DCA. and 0.55 ± 0.09 l/min placebo was not significantly different between the two conditions.

The group mean ± SEM off-transient $\dot{V}O_2$ responses are shown in figure 39. The responses for both trials are well matched. This is evident in values for the off transient time constants which are not significantly different (Mean ± SEM. 28.2 ± 2.34 s DCA., 28.7 ± 2.14 s placebo. P = 0.089. Table 8). The time constant for the off transient responses of both groups were significantly longer than the corresponding on transient responses (Mean ± SEM. 28.2 ± 2,34 s DCA off-τ. 18.3 ± 1.22 s DCA on-τ. 28.7 ± 2.14 s placebo off-τ. 16.1 ± 2.02 s on-τ. P<0.05).

Figures 40 a&b show mono-exponential fits for the off transient $\dot{V}O_2$ responses for all subjects. Once again the initial 'cardiodynamic' phase (~25 s) of the response was excluded and the fit was continued through at least one minute of the steady-state

response. Generally, the off-transient steady-state response was inherently more 'noisy' than the on-transient response. This is likely due to the weight of the subject on the saddle of the ergometer becoming more apparent to the subjects during the transition from a high load to a lighter load. Furthermore, during unloaded pedalling their breathing tended to become less 'rhythmical' as they attempted to relax in anticipation of the next step up in work rate. Therefore the fitting range was continued throughout the steady-state in order to maximise the number of data points used to determine the steady-state asymptote. The responses were generally well described by equation 20; that is the associated residuals fluctuated closely and randomly around zero.

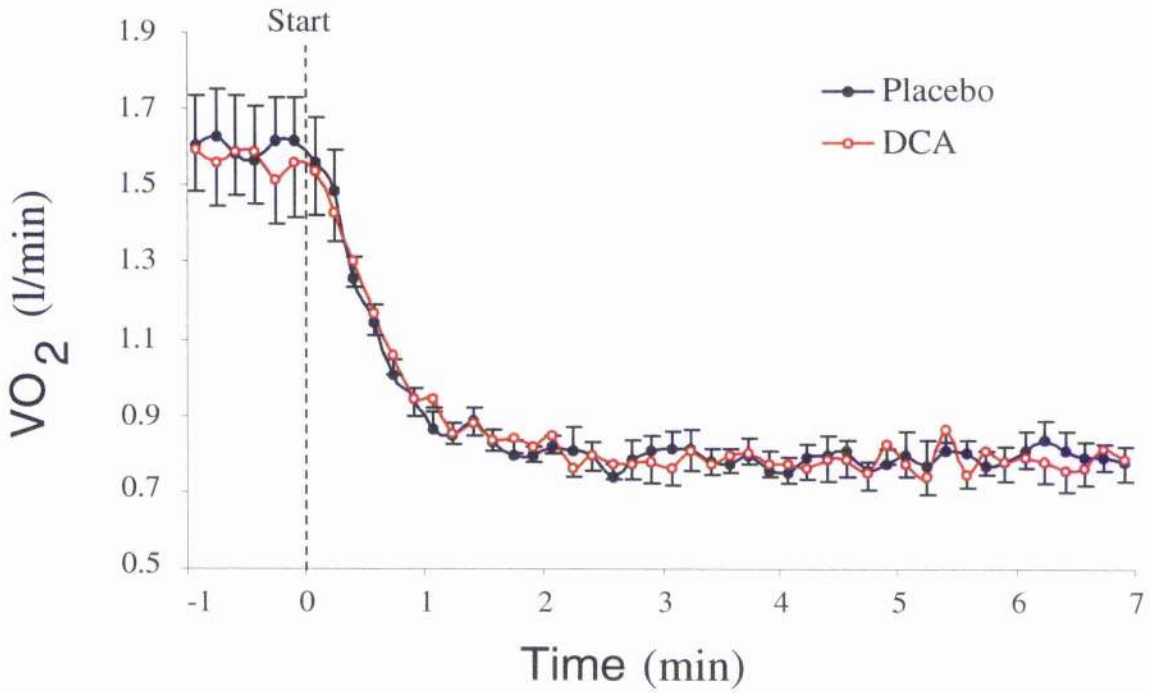


Figure 39 – Data are group mean ± SEM. The on-transient $\dot{V}O_2$ response to moderate square wave exercise. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

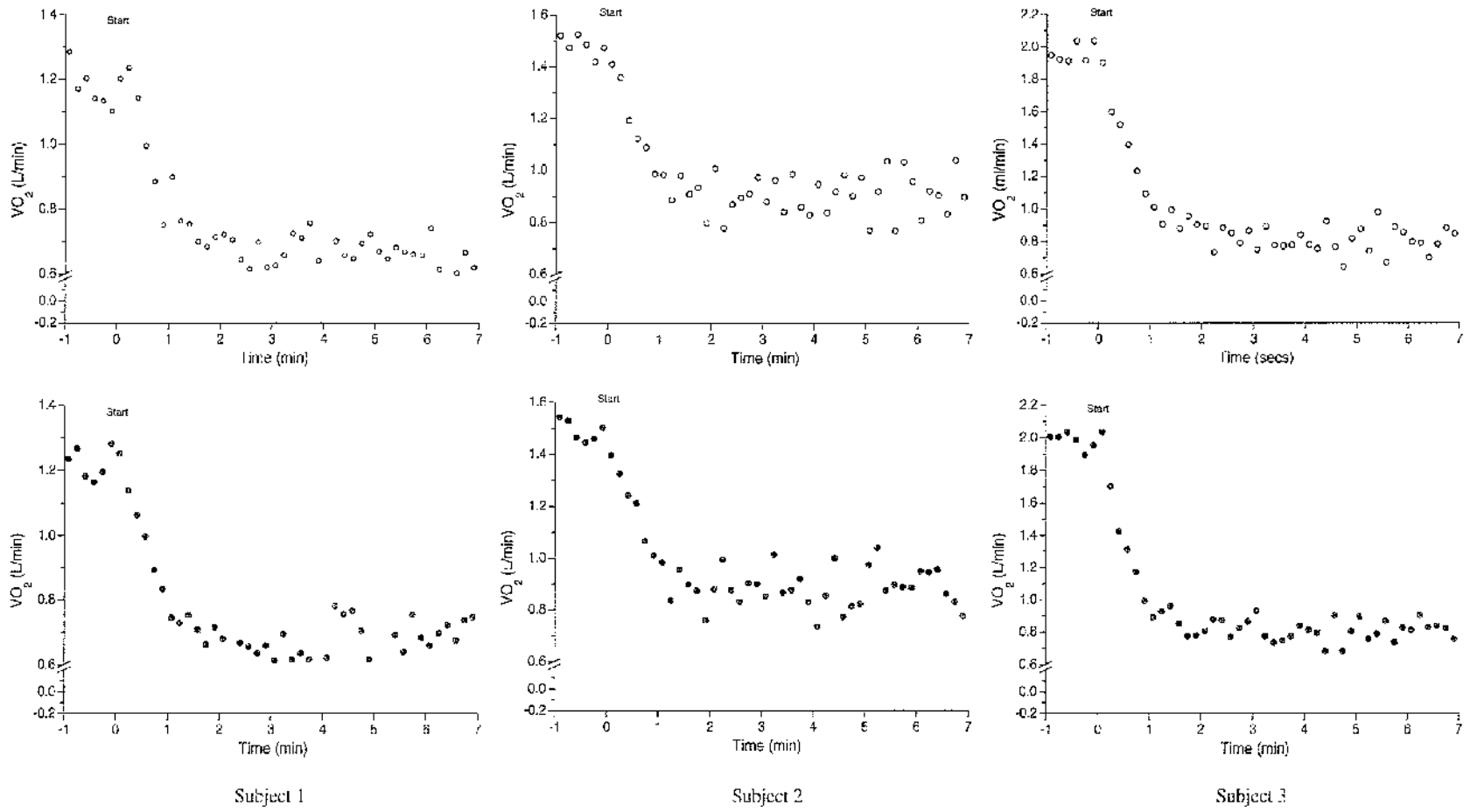


Figure 40a – Off- transient $\dot{V}O_2$ response to moderate square wave exercise. Individual plots. Subjects 1-3. Open Circles = DCA (top panels). Closed circles = placebo (bottom panels). The solid line through the data points represents the mono- exponential fit to the data. The fit began at least 20 s into exercise to exclude the cardiodynamically mediated 'phase 1' response and continued throughout the 'phase 3' steady-state response. The goodness of fit is illustrated by the fluctuations of the residuals around zero. The dashed line indicates the start of the stepped increase in work rate.

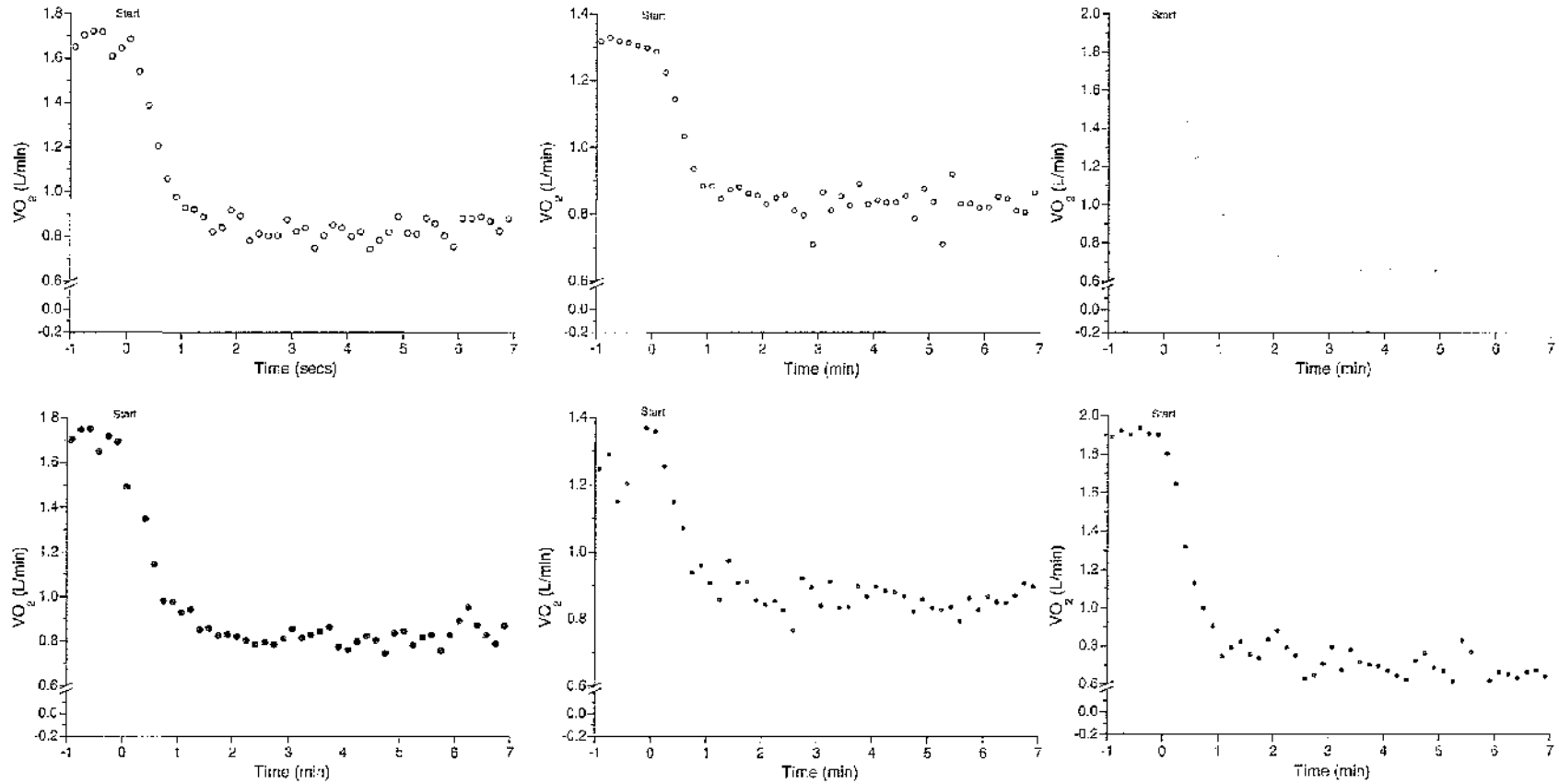


Figure 40b – Off- transient $\dot{V}O_2$ response to moderate square wave exercise. Individual plots. Subjects 4-6. Open Circles = DCA (top panels). Closed circles = placebo (bottom panels). The solid line through the data points represents the mono- exponential fit to the data. The fit began at least 20 s into exercise to exclude the cardiodynamically mediated 'phase 1' response and continued throughout the 'phase 3' steady-state response. The goodness of fit is illustrated by the fluctuations of the residuals around zero. The dashed line indicates the start of the stepped increase in work rate.

4.2.3 Cardiovascular responses to moderate intensity exercise

Resting heart rate was significantly elevated on average by 10 beat·min^{-1} in the DCA trial (Mean ± SEM. 68 ± 3.2 beat·min^{-1} DCA. 58 ± 2.3 beat·min^{-1} placebo. P = 0.017. Table 9). This response was consistent for all subjects (Table 9). Heart rate continued to be significantly elevated during baseline low load pedalling (Mean ± SEM. 87 ± 3.4 beat·min^{-1} DCA. 80 ± 3.4 beat·min^{-1} placebo. P = 0.002. Table 9) and steady-state exercise (Mean ± SEM. 111 ± 3.2 beat·min^{-1} DCA. 107 ± 2.7 beat·min^{-1} placebo. P = 0.043. Table 9).

The magnitude of the difference between the two conditions proved to be smaller at the end of the exercise when compared to unloaded pedalling values; 4 beat·min^{-1} versus 7 beat·min^{-1} respectively. Likewise the difference was smaller for unloaded pedalling compared to rest; 7 beat·min^{-1} versus 10 beat·min^{-1}. This indicates that the elevation in heart rate due to DCA administration became progressively less as the work rate increased. This is illustrated in figure 41. The heart rate can be seen to be clearly elevated at more at baseline than at end exercise. Figure 42 shows the delta heart rate responses for all subjects. A lower delta heart rate requirement on commencement of exercise is clearly evident.

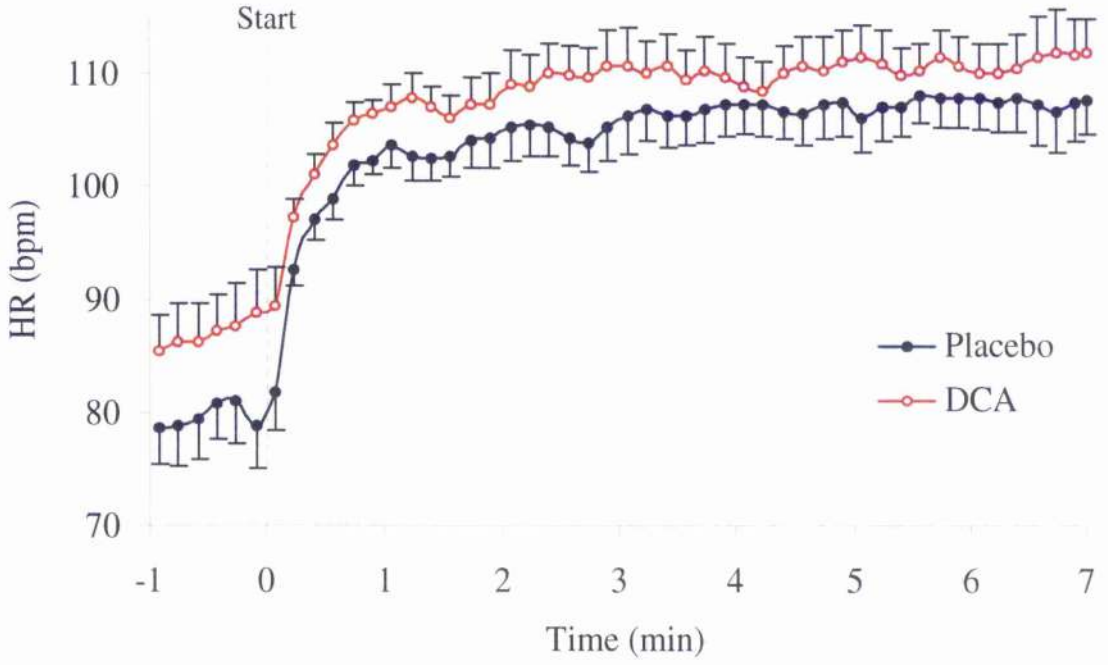


Figure 41 – Heart rate response to moderate 'square wave' exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

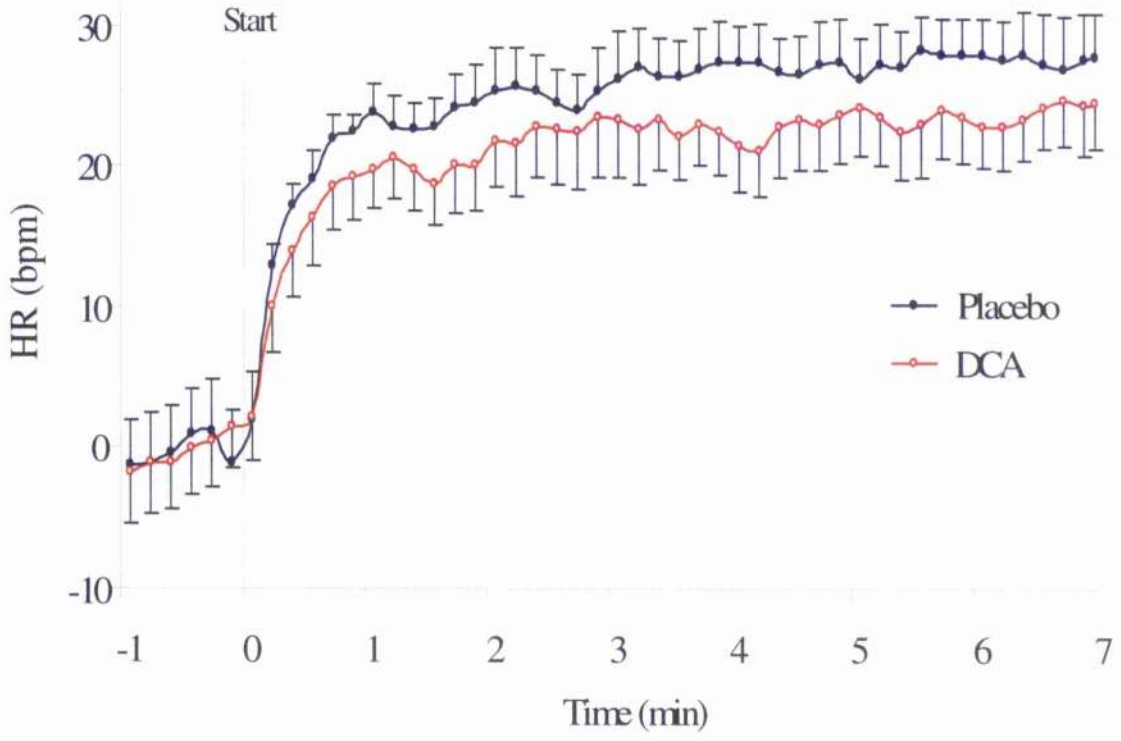


Figure 42 – Heart rate response to moderate 'square wave' exercise, normalised to baseline values. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

Table 9 - Cardiovascular responses to moderate square wave exercise

Subject	HR_{rest} (bpm)		HR_{20} (bpm)		HR_{ex} (bpm)		O_2 $Pulse_{rest}$ (ml / beat)		O_2 $Pulse_{20}$ (ml / beat)		O_2 $Pulse_{ex}$ (ml / beat)	
No.	Plac.	DCA	Plac.	DCA	Plac.	DCA	Plac.	DCA	Plac.	DCA	Plac.	DCA
1	-	-	86	92	109	113	-	-	7.95	6.70	10.87	10.37
2	63	65	80	82	98	98	8.49	6.92	10.45	10.35	15.49	15.30
3	57	64	73	80	111	114	7.77	7.44	10.88	9.62	18.16	16.92
4	62	74	84	92	117	121	-	-	9.80	9.02	14.35	13.80
5	61	77	89	98	106	114	6.49	5.49	9.18	8.35	12.70	11.23
6	49	58	67	77	103	107	9.22	7.22	9.47	8.33	18.35	16.72
Mean	58	68*	80	87*	107	111*	8.00	6.77*	9.62	8.73*	14.99	14.06*
SEM	2.3	3.2	3.4	3.4	2.7	3.2	0.48	0.36	0.42	0.51	1.21	1.13
P value		0.017		0.002		0.015		0.043		0.004		0.012

Data are individual values. Group mean ± SEM data shown. * Statistically significant between DCA and placebo trials. Statistical analysis was carried out using a paired *t*-test. Statistical significance was declared at $P<0.05$. LL = Low load pedalling (20 W). Ex = exercise. HR = Heart rate. O_2 Pulse = oxygen uptake / heart rate. – Data not available.

Oxygen pulse is significantly lower at rest in the DCA condition when compared to the placebo condition (Mean ± SEM. 6.77 ± 0.36 ml/beat DCA. 8.00 ± 0.48 ml/beat placebo. P = 0.043. Table 9). The summary data for each individual are shown in table 9. Oxygen pulse continued to be significantly reduced during low load pedalling (Mean ± SEM. 8.73 ± 0.51 ml/beat DCA. 9.62 ± 0.2 ml/beat placebo. P = 0.004. Table 9) and at end exercise (Mean ± SEM. 14.06 ± 1.13 ml/beat DCA. 14.99 ± 1.21 ml/beat placebo. P = 0.012. Table 9), in the DCA trial.

Group mean ± SEM oxygen pulse responses for both conditions are shown in figure 43. A reduction is evident throughout the DCA trial. This reflects the observed increase in heart rate with no change in $\dot{V}O_2$. Figure 44 shows the oxygen pulse responses normalised to baseline values. The time course and magnitude of the change in oxygen pulse is shown to be similar for both conditions.

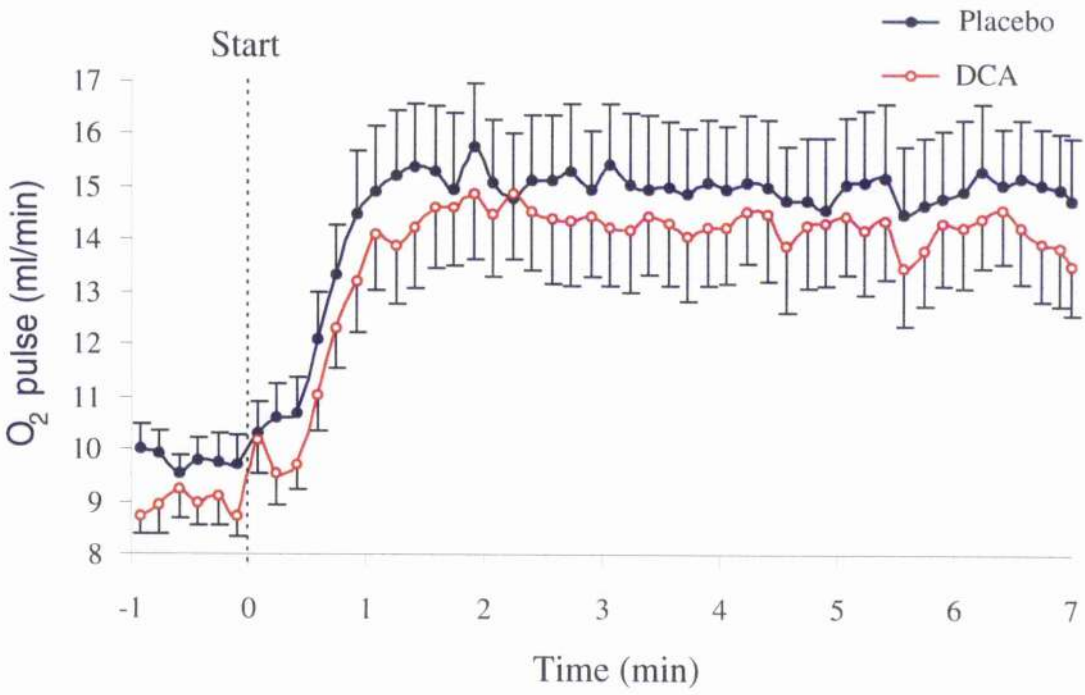


Figure 43 – Oxygen pulse response to moderate 'square wave' exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

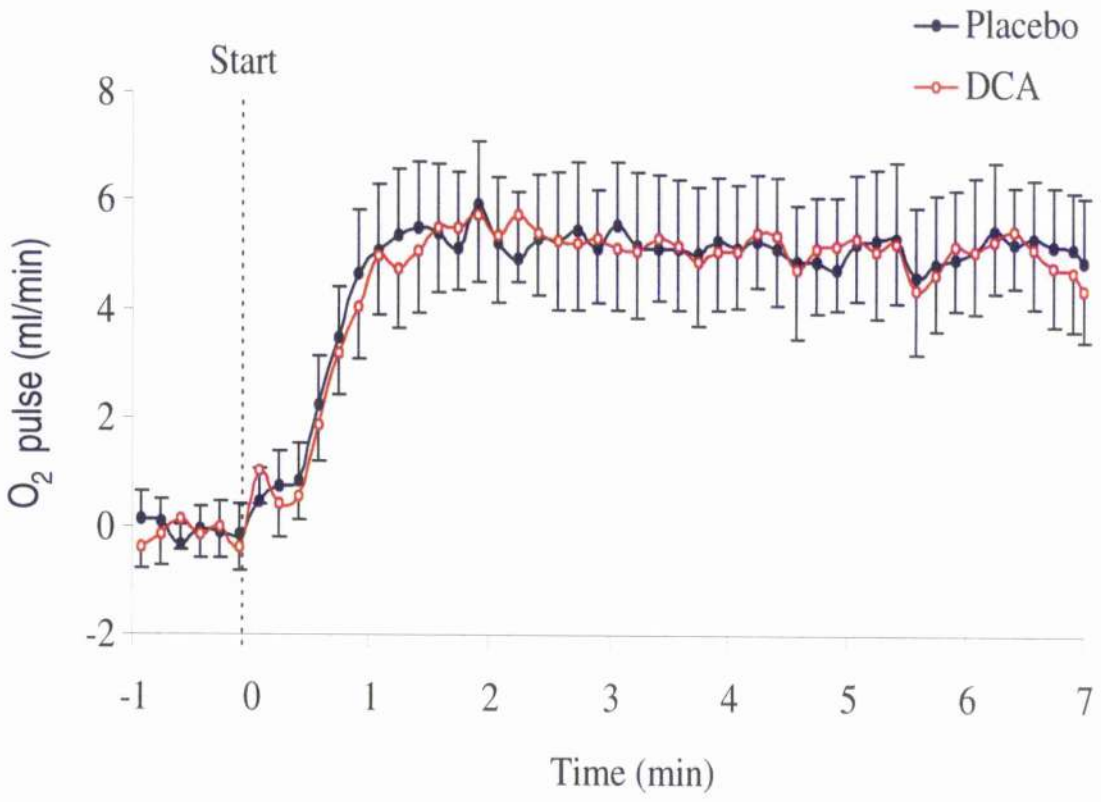


Figure 44 – Oxygen pulse response to moderate 'square wave' exercise, normalised to baseline values. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

4.2.4 Muscle oxygen saturation responses to moderate intensity exercise

Group mean ± SEM deoxyhaemoglobin responses for both conditions are shown in figure 45. All data are corrected to a % of a maximal voluntary contraction and normalised to the last minute of the low load baseline. The magnitude of the delta is reduced during the DCA trial. Figure 46 shows individual responses for all subjects. It is clear from this figure that a lower delta is not consistent for all subjects. The magnitude of the drop in subjects 1 and 2 during the DCA trial clearly offsets the mean for this group.

Across all subjects there was a rapid drop in Hb during the first ~25 s of exercise. Taken as a group mean this drop appears greater during the DCA trial. This may indicate a greater rate of oxygen utilisation during this initial phase of exercise. A response that would be masked in the 'cardiodynamic' phase of the $\dot{V}O_2$ response. Alternatively, and perhaps more likely, this may indicate a greater increase in the size of the blood volume under the field of study. This would cause a greater drop in the Hb signal during this time. This response is not consistent across all subjects (figure 46). The resolution of the responses in this phase is not high and therefore this finding may warrant further investigation.

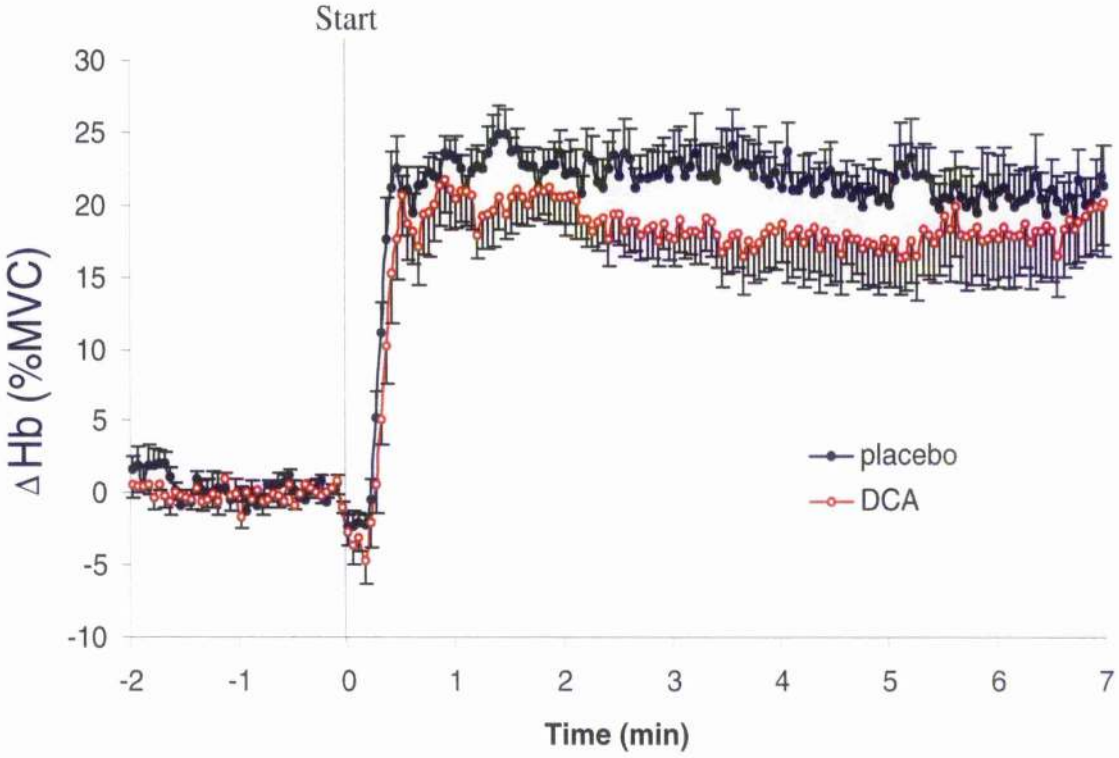


Figure 45 - Delta deoxyhaemoglobin responses to moderate 'square wave' exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

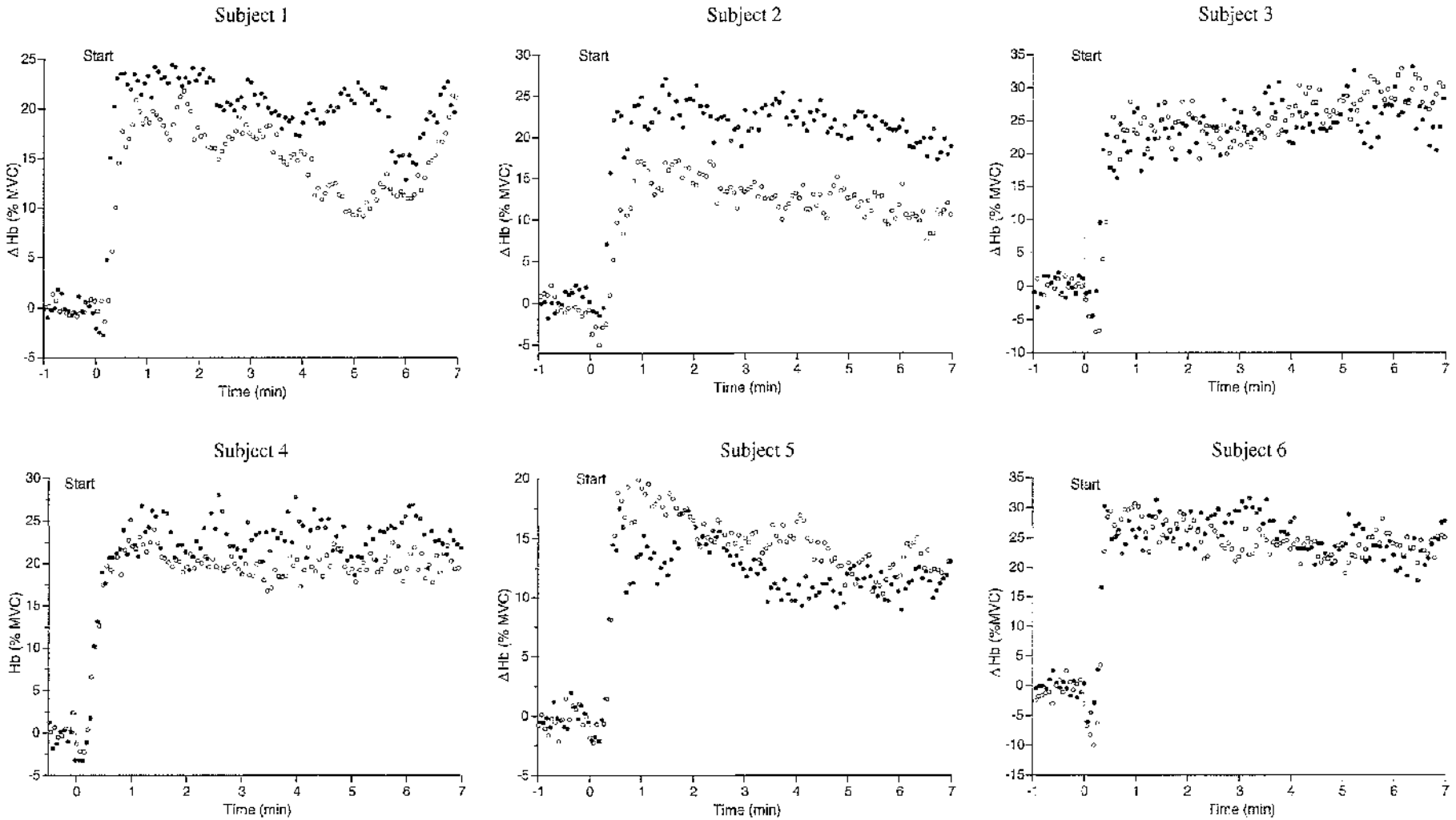


Figure 46 – Delta deoxyhaemoglobin responses to moderate square wave exercise. Individual plots overlaid. Open circles = DCA. Closed circles = placebo. Subjects 1-6. The dashed line indicates the start of the stepped increase in work rate from a 20 W baseline.

4.2.5 Ratings of perceived exertion and dyspnoea during moderate intensity exercise

Figures 47 and 48 illustrate the comparative group means ± SEM for ratings of perceived exertion and ratings of dyspnoea for the two conditions. No significant difference was found between visits for either measure. Interestingly, there was a tendency to rate both dyspnoea and RPE higher on the second exercise bout than the first. This may reflect general discomfort with the setup and prolonged use of the mouthpiece. However, these differences did not reach statistical significance.

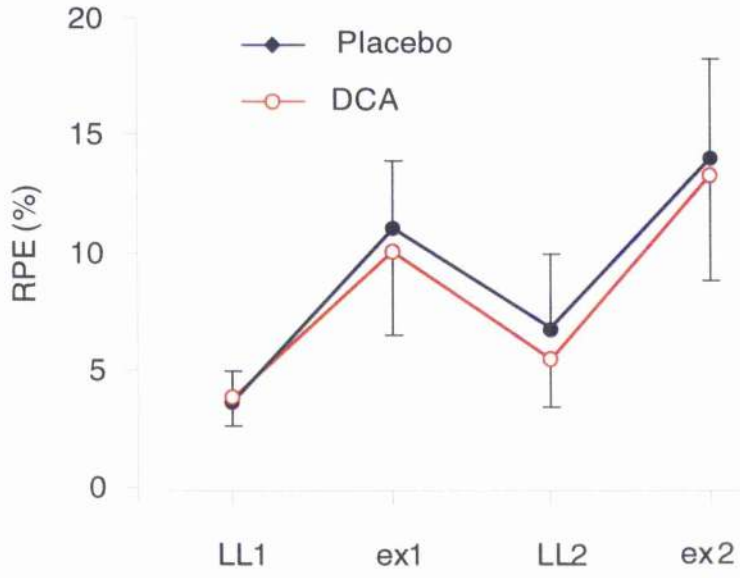


Figure 47 - Ratings of perceived exertion during moderate intensity exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. LL = low load pedalling (20 W). Ex = exercise sample.

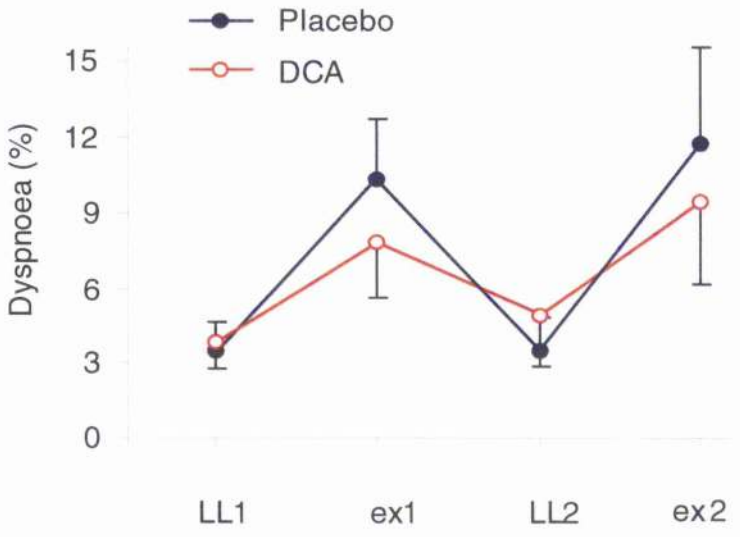


Figure 48 - Ratings of dyspnoea during moderate intensity exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. LL = low load pedalling (20 W). Ex = exercise sample.

Chapter 4

4.3 Discussion

4.3.1 The effect of DCA on oxygen uptake during moderate intensity exercise

The ~50% reduction in blood lactate concentration seen throughout exercise in this study is consistent with that reported in the literature (Roberts *et al.*, 1997; Ludvik *et al.*, 1993; Timmons *et al.*, 1998; Ludvik *et al.*, 1991; Carraro *et al.*, 1989; Howlett *et al.*, 1999a&b; Wilson *et al.*, 1988; Rossiter *et al.*, 2003). This response provides evidence that the DCA preparation was successful in activating PDH (Stacpoole, 1989; Timmons *et al.*, 1998; Howlett *et al.*, 1999a&b; Gibala and Saltin, 1999; Parolin *et al.*, 2000).

This apparent increase in PDH activity was not associated with any significant speeding of the $\dot{V}O_2$ on kinetics. Indeed the $\dot{V}O_2$ steady state or kinetic responses show little variation between trials. As expected this is borne out in the calculated values for the O_2def (0.59 $\pm$ 0.26 DCA vs 0.55 $\pm$ 0.21 placebo l/min). Therefore, this study does not support the suggestion by Timmons *et al* (1998), and Greenhaff *et al* (2003), that an acetyl-group deficit limits mitochondrial ATP production at the onset of exercise.

Timmons *et al* (1998), Howlett *et al* (1999), Parolin *et al* (2000) and more recently Rossiter *et al* (2003) have all demonstrated a significant reduction in PCr breakdown and blood lactate accumulation in response to DCA administration during constant load exercise (supra-threshold exercise intensity). In the study by Rossiter *et al* (2003) these responses were accompanied by a reduction in the fundamental $\dot{V}O_2$ amplitude by ~8% with no associated change in the fundamental τ. The suggestion from these

investigators is that the reduction in PCr degradation on DCA administration reflects an improved energy state of the muscle that is not associated with an altered time constant for $\dot{V}O_2$. The evidence from the above studies is derived from supra-threshold exercise. However, the fundamental response has been argued by Margaria *et al* (1965), Paterson & Whipp (1992) and Ozyener *et al* (2001) to be similar in nature to that for sub-threshold exercise. Therefore comparison of the fundamental response from this study and the results of Timmons *et al* (1998), Howlett *et al* (1999), Parolin *et al* (2000) and Rossiter *et al* (2003) is not unfounded.

The data from this study does not agree with that of Rossiter *et al* (2003) in that the steady state gain in $\dot{V}O_2$ was unaffected by DCA administration (0.81 $\pm$ 0.33 l/min DCA vs 0.83 $\pm$ 0.33 Placebo l/min). A reason for this difference could be that while the fundamental response below and above the lactate 'threshold' are similar in their description they are not wholly governed by the same mechanism/s of control. For instance, factors such as proportional recruitment of muscle fibre type and changes in pH could play more of a role in setting the fundamental $\dot{V}O_2$ gain above the threshold than below. Indeed, Burnley *et al* (2001) reported that prior heavy exercise increases the amplitude of the primary $\dot{V}O_2$ component during supra-threshold exercise. However, the same group had previously shown no effect of prior heavy exercise on the fundamental $\dot{V}O_2$ response to moderate exercise (Burnley *et al*, 2000).

4.3.2 Parameter discrimination

The small number of subjects in this study reduces the statistical power of the results. Howlett & Hogan (2003) found a ~ 2 s increase in the rate of fall in PO_2 using DCA in an isolated animal muscle preparation. Using the standard deviation in τ from this study; a retrospective power analysis indicates that the number of subjects required to realise a significant change of 2 s in τ is 17. Therefore this study is underpowered. This result becomes less critical as the tendency was for τ to be lengthened in the DCA trial. Therefore a significant speeding of the kinetic response was unlikely to be seen even with greater numbers.

4.3.3 The effect of DCA on muscle oxygen saturation during moderate intensity exercise

Delorey *et al* (2003) and Shibuya *et al* (2003) have attempted to relate the change in NIRS-Hb and $\dot{V}O_2$ in response to moderate intensity exercise. Both studies modelled the deoxy-haemoglobin response to moderate constant work exercise with a mono-exponential function. They found the on-kinetics for ΔHb to be faster than $\dot{V}O_2$ kinetics (6.5 $\pm$ 2.2 s vs 23.8 $\pm$ 4.4 s, Delorey *et al.*, 2003; 10.0 $\pm$ 3 s vs 30 $\pm$ 8 s Shibya *et al.*, 2003). The ΔHb plots from this study were not expressed well by a monoexponential function, largely due to an inconsistent steady-state phase. However, visual inspection of the ΔHb time plots clearly show that the results from this study agree with those of Delorey *et al* (2003) and Shibya *et al* (2003) with the ΔHb response increasing more rapidly than the $\dot{V}O_2$ response. The ΔHb signal is an estimate of changes in muscle microvascular deoxygenation within the field of interrogation and

reflects a balance between local muscular O_2 delivery and muscle oxygen utilisation. The hypothesis from this and the above studies was that the phase II $\dot{V}O_2$ response would reflect the NIRS-Hb response. That is, after an initial delay, the NIRS-Hb signal would increase following a mono-exponential time course to a steady-state. As with the results of Delorey *et al* (2003), a clear delay of ~15 s between the start of transition and the increase in ΔHb is evident. However in contrast to the studies of Shibya *et al* (2003) and Delorey *et al* (2003), where ΔHb remained constant during this period, the results of this study show a clear decrease in the Hb signal during the initial phase of the response. It is likely that this initial drop in the ΔHb signal is evidence of either an increased perfusion of the measurement field or an initial movement of Hb out of this field. The latter suggestion could possibly be due to the actions of the muscle pump at the onset of the exercise transition.

The rate of change of the ΔHb signal reflects the balance between muscle O_2 delivery and muscle O_2 utilisation. This initial drop in the ΔHb signal could reflect a more rapid increase in muscle blood flow relative to muscle O_2 extraction. This would suggest that delivery of O_2 is not limiting during this initial phase of exercise. The time delay before muscle O_2 extraction becomes evident agrees with the work of Grassi *et al* (1996) and Bangsbo *et al* (2000) in intact human muscle and the work of Hogan (2001) in Xenopus single skeletal muscle fibres. These authors report a ~6-20 s delay after the start of muscle contraction before O_2 extraction was evident. This 'apparent' delay in microvascular O_2 extraction may be due to a 'metabolic inertia' within the oxidative machinery of the mitochondria or it may reflect a matching of O_2 extraction by local muscular O_2 stores and muscle O_2 delivery. However, the evidence from studies utilising magnetic resonance spectroscopy (Whipp *et al.*, 1999; Rossiter *et al.*, 1999)

show PCr decreasing immediately at exercise onset. Furthermore, the work by Behnke *et al* (2002) shows that measures of microvascular PO_2 also decrease without delay. These studies do not support the existence of an inherent 'metabolic inertia' within the machinery of oxidative phosphorylation. Delorey *et al* (2003) suggest that the constant ΔHb signal during the first ~13 s of exercise in their study reflects a close matching of perfusion and metabolism and that any delay in O_2 consumption should be reflected by a decrease in ΔHb during this initial phase. Therefore, the results from this study appear to support the notion of a delay in O_2 consumption at the start of exercise. Whether the early decrease in ΔHb evident in this study reflects a 'metabolic inertia' or an increase in muscle perfusion/oxygen extraction ratio cannot be determined.

DCA has been shown to decrease peripheral vascular resistance (Wargovich *et al.*, 1988; Ludvik *et al.*, 1991). This may augment muscle blood flow during the initial stages of the exercise response. Therefore a reduction in the ΔHb signal at the start of exercise may be expected. There is a clearly exaggerated decrease in the ΔHb signal for 3 of the 6 subjects during the DCA trial in this study. However, no difference is seen for the remaining 3 subjects. Therefore, DCA has had no consistent/if any effect on ΔHb during this phase of the response. A DCA induced increase in muscle perfusion may be expected to reduce the ΔHb response throughout the exercise bout. Again this is evident in only 3 of the 6 subjects. Generally there is no systematic difference between the ΔHb responses for the DCA and Placebo trials. The responses for all subjects reach a peak value within 1 min, evidently quicker than the $\dot{V}O_2$ response. By definition a steady state response is not met in the majority of subjects. This clearly does not reflect the $\dot{V}O_2$ response and differs from the results of Delory *et al* (2003) and Shybia *et al* (2003). Both studies reported a mono-exponential response to steady-state.

Closer inspections of the data from the Delory *et al* (2003) study suggest their description of the response is not accurate. The individual plots clearly show a 'drifting' in the 'steady state' portion of the response. Shybia *et al* (2003) do not display the ΔHb response in their study. The drifting is cleary evident in the results from this study and likely reflects factors influencing the volume of Hb in the region of interrogation.

The results from this study suggest that DCA has no systematic effect on microvascular O_2 extraction during sub-threshold exercise. There may be a suggestion that DCA increases muscle perfusion during the initial phase of the response, but there is clearly no effect on the point of increase in ΔHb and no evident speeding of the response in 4 out of the 6 subjects.

4.3.4 The effect of DCA on heart rate during moderate intensity exercise

As with the incremental exercise study described in chapter 3 of this thesis, the heart rate was elevated at rest and unloaded pedalling during the DCA trial. Unlike the incremental exercise, heart rate was elevated throughout the moderate exercise transitions. The effect of DCA on heart rate will be discussed further in the general discussion (section 6.8). The delta heart rate plots from this study clearly show that the effect of DCA on heart rate during exercise is not simply additive. The difference in heart rate between the two trials is evidently reduced on exercise.

The result of an elevated heart rate is a reduction in the oxygen pulse. Oxygen pulse is a product of stroke volume and arterio-venous O_2 difference. Therefore a reduction in oxygen pulse can reflect either a reduced stroke volume, an increased oxygen delivery

or a decreased oxygen extraction. The increased heart rate during the DCA trial reduces the O_2 pulse, presumably through changes in stroke volume. This contradicts previous discussion (see section 3.3) suggesting that DCA may play a role in increasing O_2 delivery by increasing cardiac output. However when the O_2 pulse plots have been normalised to a zero baseline it is evident that there is little change in the rate or magnitude of the O_2 pulse response. This suggests that there is little change in either stroke volume or arterio-venous O_2 difference as a result of DCA administration during exercise at moderate work intensity.

Chapter 5

The effect of Dichloroacetate on oxygen uptake during 'heavy' intensity exercise

Chapter 5

5.0 Introduction

The hypotheses for this study were that DCA could alter the $\dot{V}O_2$ response to 'heavy'/ 'very heavy' exercise in three ways: 1) by speeding the initial 'fundamental' component of the response 2) changing the gain of the initial 'fundamental' component of the response 3) by reducing the amplitude of the slow component response.

The reasoning for the first hypothesis is similar to that described for sub-threshold exercise (section 3.0). Timmons *et al* (1998) described a 'functional stenosis' at the level of pyruvate dehydrogenase that was considered to be 'major determinant' of the lag in oxygen uptake during the initial stages of exercise. This was supported by the results from subsequent studies by Howlett *et al* (1999) and Parolin *et al* (2000). On the evidence from these studies, DCA administration should result in a speeding of the initial 'fundamental' $\dot{V}O_2$ response.

In addition, studies have shown an apparent speeding of $\dot{V}O_2$ kinetics for exercise transitions completed from a background of prior exercise (Gerbino *et al.*,1996; MacDonald *et al.*, 1997; Bohnert *et al.*, 1998). It has been proposed that this speeding of the $\dot{V}O_2$ response is due to a residual acidosis augmenting the oxygen delivery during the subsequent exercise bout (Gerbino *et al.*, 1996). However, PDH is quickly activated during exercise (Putman *et al.*, 1998). Therefore it is conceivable that a prior bout of exercise effects a priming of PDH, augmenting the $\dot{V}O_2$ response to the subsequent bout of exercise. This is an effect similar to that described by Timmons *et al* (1998). Indeed Campbell-O'Sullivan *et al* (2002) reported a prior bout of low intensity exercise (55%

$\dot{V}O_{2\,max}$) accelerated the $\dot{V}O_2$ on-kinetic response over the first minute of a subsequent higher intensity (75% $\dot{V}O_{2\,max}$) exercise bout. This increase in speed of the $\dot{V}O_2$ on-kinetic response was attributed to an increase in the acetyl group availability at the level of the TCA cycle, thereby accelerating mitochondrial ATP production at the onset of the second exercise bout. Therefore it is conceivable that DCA administration should exhibit similar effect on the $\dot{V}O_2$ on-kinetic response to supra threshold exercise in the 'heavy' and 'very heavy' exercise intensity domains.

As described in the introduction (refer to section 1.11), the mechanism/s behind the manifestation of the slow component are not yet fully understood. However what is clear is that the slow component is only evident at work rates that induce lactic acidaemia and it is dependent on a relative work rate rather than an absolute work rate. While blood lactate is unlikely to be a primary cause of the $\dot{V}O_2$ excess, these two components of the exercise response appear inextricably linked. This is particularly apparent as a result of prior exercise (Gerbino *et al.*, 1996) and endurance training (Casaburi *et al.*, 1987). It was of interest therefore to investigate whether DCA, and subsequently PDH activation, exhibited similar characteristics. That is, would a reduction of the anaerobic contribution to the fundamental phase of the exercise response alter the relative intensity of the exercise bout, and subsequently reduce the $\dot{V}O_2$ excess? In addition, is a greater lactate clearance, and a subsequent reduction in acid stress, responsible for a reduction in the slow component?

Chapter 5

5.1 Methods

5.1.1 Subjects

7 men between the ages of 21 and 35 took part in the protocol. All subjects met the standard inclusion criteria (section 2.1 general methods). Table 10 shows the subject characteristics for each individual.

Table 10 - Subject characteristics

Subject	Height (cm)	Body mass (kg)	Age (yrs)
1	179	61	22
2	182	95	33
3	184	87	35
4	167	71	28
5	170	83	25
6	186	86	22
7	181	67	21
Mean	178	78	27
SD	7	12	6

5.1.2 Protocol

Prior to undertaking this study subjects were familiarised with all equipment and procedures during multiple visits to the laboratory. On two subsequent visits the subjects completed a single square wave exercise protocol until exhaustion or until they were no longer able to maintain a cadence of 50 rpm. After 3 min of resting breathing and 5 min of low load pedalling at 20 W, the work rate was increased (fig. 49). Up to 10 min of data was collected during the recovery period post exhaustion.

Work rates above the lactate threshold were calculated as a percentage of delta $\dot{V}O_2$. Delta $\dot{V}O_2$ is a term used to define the $\dot{V}O_2$ difference between $\dot{V}O_{2\,max}$ and $\dot{V}O_2$ at the lactate threshold i.e. $\dot{V}O_{2\,max} - \dot{V}O_{2LT}$ (Rausch *et al*, 1991). The percentage of delta $\dot{V}O_2$ used was determined from the familiarisation bouts. Typically 60% of delta $\dot{V}O_2$ was used for exercise lasting approximately 20 min. DCA and placebo doses were administered following a standard protocol (see section 2.2.5) to each visit in a double-blind, randomised fashion.

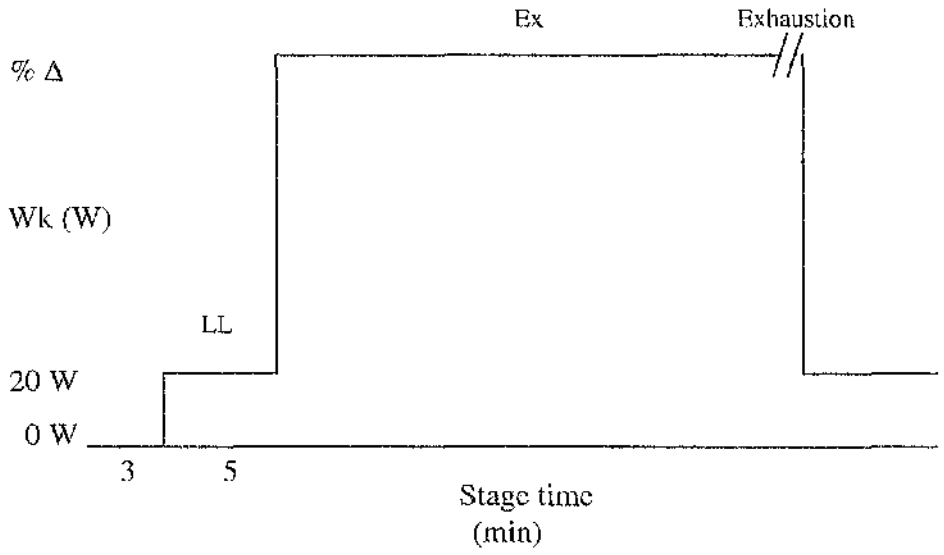


Figure 49 - Schematic illustrating the Supra-threshold exercise protocol . LL = Low Load pedalling. Ex = exercise. The exercise workload was set at approximately 60% of delta $\dot{V}O_2$ ($\Delta\dot{V}O_2 = \dot{V}O_{2\,max} - \dot{V}O_{2LT}$) for each individual. The solid line illustrates the square wave nature of the work rate increase.

Blood handling and analysis followed the standard procedures described in section 2.2.4. Blood samples were taken prior to DCA administration, 4 min into low load pedalling, every 5 min during exercise, at exhaustion and 3 min, 6 min and 9 min post exhaustion. Ratings of perceived exertion and dyspnoea were taken 45 s before each blood sample and as close to exhaustion as possible. Heart rate, gas exchange, and NIRS measurements were made as described in chapter 2.

5.1.3 Analysis

Each $\dot{V}O_2$ data set was edited as described by Rossiter *et al* (2000). Gaussian distribution characteristics were assumed (Lamarra *et al.*, 1987; Rossiter *et al.*, 2000). The $\dot{V}O_2$ response for each test was interpolated on a second-by-second basis. This allowed subsequent averaging (8-12 s) and comparison of the responses from repeated tests. The first 3 min of the $\dot{V}O_2$ response was modelled using the same mono-exponential function as that used for the sub threshold data (*Eqn 20*).

The initial 'cardiodynamic' portion of the data was excluded from the fit. The 3 min time point was chosen to standardise the fitting window for comparison between the two trials. 3 min was chosen to maximise the data set available for analysis while minimising the contribution of the slow component response associated with exercise above the lactate threshold. The initial portion of the slow rise in $\dot{V}O_2$ was described by subtracting an average of minute 3 from the average of minute 6 to give the index $\dot{V}O_{2\ 6\text{-}3}$. Peak $\dot{V}O_2$ was taken as an average of the last 20 s of exercise.

This analysis was adopted as the standard approach. Multiple exponential functions, as described in the general introduction, were unsuccessful in consistently describing the $\dot{V}O_2$ response for each individual.

5.1.4 Statistics

Statistical analysis followed that described in general methods chapter 2 (section 2.2.11)

Chapter 5

5.2 Results

5.2.1 Blood borne metabolic responses to supra-threshold exercise

The group mean ± SEM blood lactate responses for both trials are shown in figure 50. It is clear that blood lactate was reduced throughout exercise on the DCA trial. This reduction was significant at baseline (Mean ± SEM. 0.35 ± 0.05 mmol/l DCA. 0.71 ± 0.0.08 mmol/l placebo. P = 0.0005. Table 11), minute 3 (Mean ± SEM. 4.04 ± 0.57 mmol/l DCA. 4.85 ± 0.59 mmol/l placebo. P = 0.0009. Table 11), minute 6 (Mean ± SEM. 8.44 ± 1.15 mmol/l DCA. 7.55 ± 1.22 mmol/l placebo. P = 0.046. Table 11) and exhaustion (Mean ± SEM. 11.82 ± 1.29 mmol/l DCA. 13.49 ± 1.55 mmol/l placebo. P = 0.008. Table 11). Blood lactate values continued to be lower during recovery. A significant difference was observed at 3 min (Mean ± SEM. 11.07 ± 1.46 mmol/l DCA. 12.65 ± 1.46 mmol/l placebo. P = 0.013. Table 11) and 6 min (Mean ± SEM. 9.77 ± 1.87 mmol/l DCA. 11.53 ± 1.83 mmol/l placebo. P = 0.036. Table 11) post exhaustion. DCA lowered blood lactate values across all time points by 51%, 11%, 9%, 12%, 12% and 15% respectively. Therefore the magnitude of the reduction became less the closer the subject was to exhaustion.

There was a small but significant fall in pH from baseline to exhaustion (fig. 51) in both the placebo trial (Mean ± SEM. 7.40 ± 0.003 baseline, 7.24 ± 0.03 exhaustion. P = 0.012. Table 11) and DCA trial (Mean ± SEM. 7.41 ± 0.002 baseline, 7.25 ± 0.03 exhaustion. P = 0.011. Table 11). However there was no difference in the magnitude of the drop between trials.

Similarly there was a fall in standard bicarbonate progressively throughout exercise until exhaustion (fig. 52). There appears to be a trend for the values to be higher on the DCA trial, the mean differences do not reach statistical significance (Summary data are shown in Table 11).

Figure 53 shows that blood glucose increased at exhaustion and peaked post exercise for both trials. There appears to be a tendency for the mean values to be lower for the DCA trial however the differences do not reach statistical significance (Summary data are shown in Table 11).

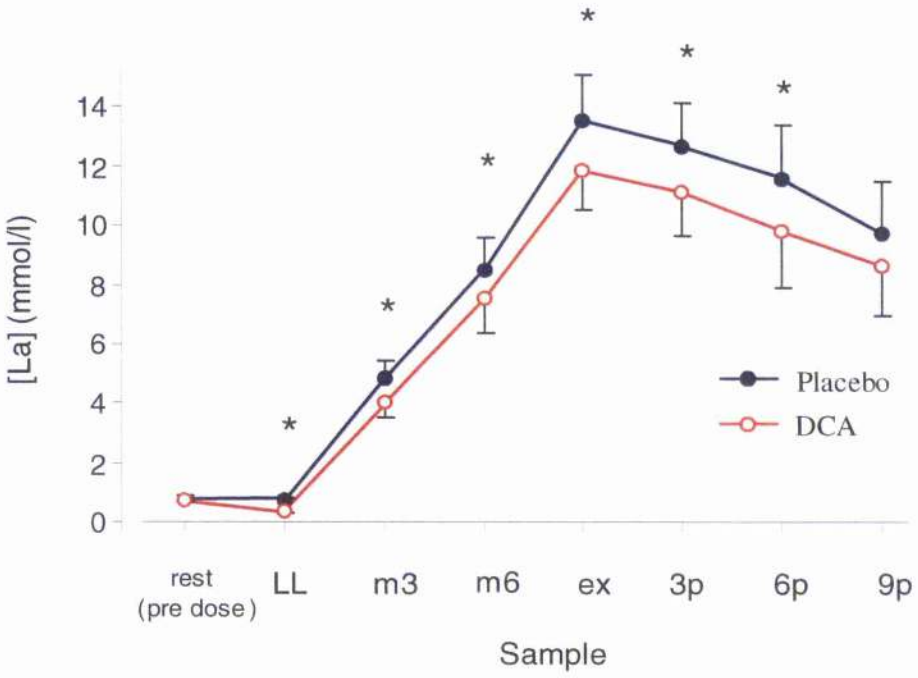


Figure 50 - The effect of DCA on blood lactate during heavy intensity exercise. Data are group means ± SEM. Closed circles = placebo. Open circles = DCA. Data shows that the administration of DCA successfully reduced blood [Lactate] throughout the supra-threshold exercise protocol, compared to the placebo trial. Pre dose = Samples taken prior to DCA administration. LL = low load pedalling (20W). m3 = minute 3. m6 = minute 6. ex = exhaustion. 3p = 3 minutes post exhaustion. 6p = 6 minutes post exhaustion. 9p = 9 minutes post exhaustion. * Statistically significant between DCA and placebo trial. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at $P < 0.05$.

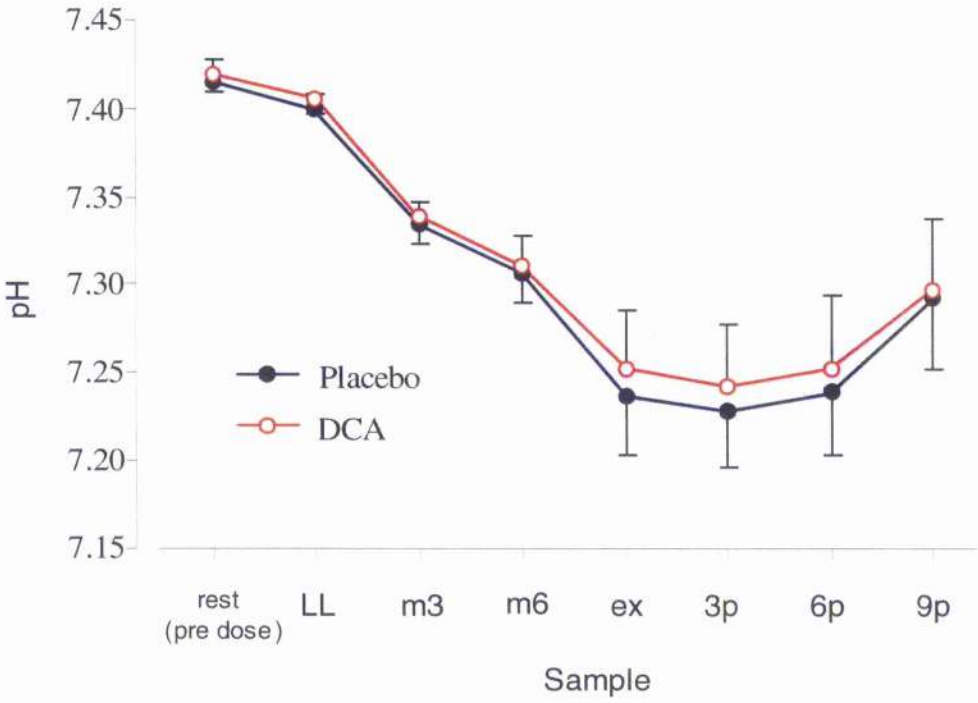


Figure 51 - The effect of DCA on blood pH during heavy intensity exercise. Data are group means ± SEM. Closed circles = placebo. Open circles = DCA. Pre dose = Samples taken prior to DCA administration. LL = low load pedalling (20W). m3 = minute 3. m6 = minute 6. ex = exhaustion. 3p = 3 minutes post exhaustion. 6p = 6 minutes post exhaustion. 9p = 9 minutes post exhaustion. * Statistically significant. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at $P < 0.05$.

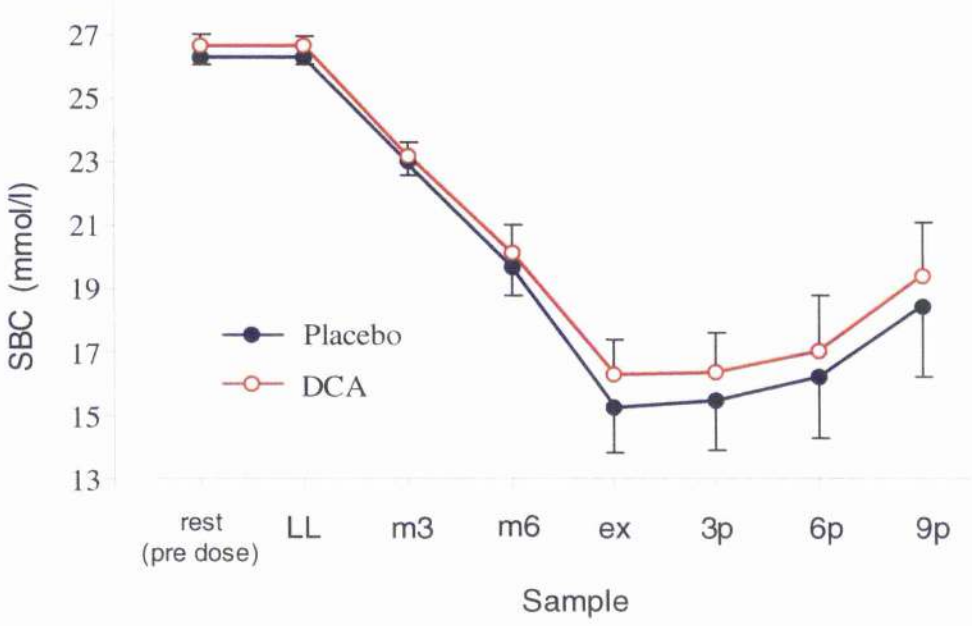


Figure 52 - The effect of DCA on blood bicarbonate during heavy intensity exercise. Data are group means ± SEM. Closed circles = placebo. Open circles = DCA. Pre dose = Samples taken prior to DCA administration. LL = low load pedalling (20W). m3 = minute 3. m6 = minute 6. ex = exhaustion. 3p = 3 minutes post exhaustion. 6p = 6 minutes post exhaustion. 9p = 9 minutes post exhaustion. * Statistically significant. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at $P < 0.05$.

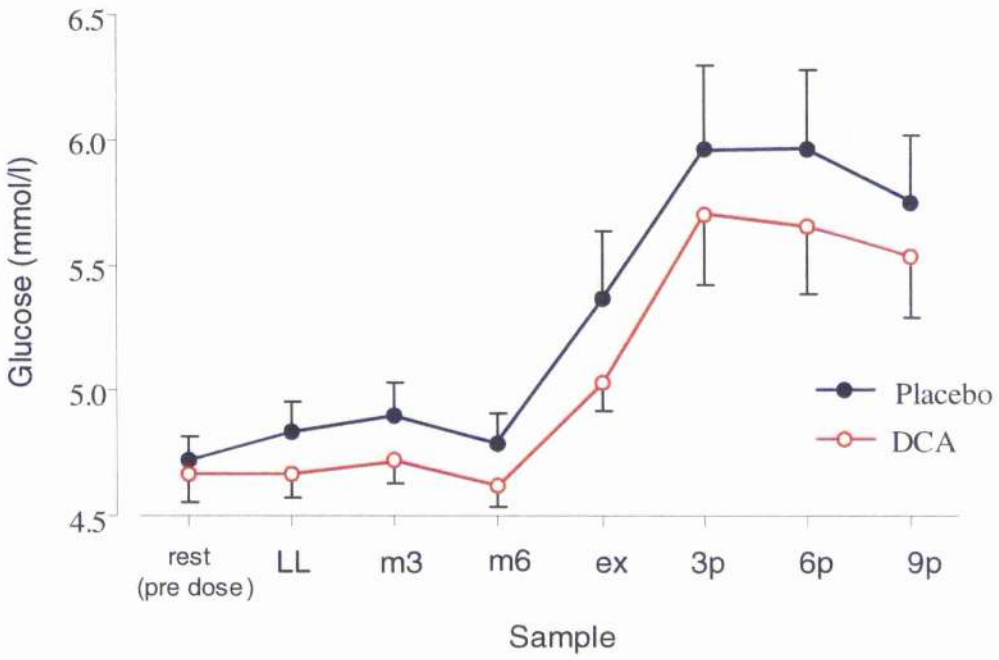


Figure 53 - The effect of DCA on blood glucose during heavy intensity exercise. Data are group means ± SEM. Closed circles = placebo. Open circles = DCA. Pre dose = Samples taken prior to DCA administration. LL = low load pedalling (20W). m3 = minute 3. m6 = minute 6. ex = exhaustion. 3p = 3 minutes post exhaustion. 6p = 6 minutes post exhaustion. 9p = 9 minutes post exhaustion. * Statistically significant. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at $P < 0.05$.

Table 11 - Blood borne metabolic responses to 'heavy' intensity exercise

	Rest (pre dose)	Baseline (20w)	3 min	6 min	Exhaustion	3min Post	6min post	9min post
La. mmol/l								
Placebo	0.72 ± 0.14	0.71 ± 0.08	4.85 ± 0.59	8.44 ± 1.15	13.49 ± 1.55	12.65 ± 1.46	11.53 ± 1.83	9.70 ± 1.79
DCA	0.73 ± 0.06	0.35* ± 0.05	4.04* ± 0.57	7.55* ± 1.22	11.82* ± 1.29	11.07* ± 1.46	9.77* ± 1.87	8.63 ± 1.72
P value	0.95	0.0005	0.0009	0.046	0.008	0.013	0.036	0.114
pH								
Placebo	7.42 ± 0.01	7.40 ± 0.003	7.33 ± 0.01	7.31 ± 0.02	7.24 ± 0.03	7.23 ± 0.03	7.24 ± 0.04	7.29 ± 0.04
DCA	7.42 ± 0.01	7.41 ± 0.002	7.34 ± 0.01	7.31 ± 0.02	7.25 ± 0.03	7.24 ± 0.04	7.25 ± 0.04	7.30 ± 0.04
P value	0.64	0.084	0.46	0.43	0.25	0.32	0.41	0.84
SBC mmol/l								
Placebo	26.31 ± 0.24	26.31 ± 0.24	23.04 ± 0.44	19.68 ± 0.87	15.26 ± 1.41	15.43 ± 1.56	16.19 ± 1.94	18.41 ± 2.22
DCA	26.64 ± 0.37	26.76 ± 0.29	23.19 ± 0.40	20.13 ± 0.89	16.27 ± 1.13	16.31 ± 1.31	17.01 ± 1.75	19.37 ± 1.75
P value	0.24	0.11	0.53	0.29	0.20	0.29	0.40	0.46
Gluc. mmol/l								
Placebo	4.72 ± 0.10	4.84 ± 0.12	4.90 ± 0.13	4.79 ± 0.13	5.36 ± 0.28	5.96 ± 0.34	5.97 ± 0.32	5.74 ± 0.27
DCA	4.67 ± 0.11	4.66 ± 0.09	4.73 ± 0.10	4.62 ± 0.08	5.03 ± 0.11	5.70 ± 0.28	5.65 ± 0.27	5.53 ± 0.25
P value	0.45	0.060	0.21	0.18	0.23	0.32	0.36	0.51

Group data (n=8). Data are mean values ± SEM. * Statistically significant between DCA and placebo trials. Statistical analysis was carried out using two-factor ANOVA for repeated measures, followed by a paired *t*-test if a main treatment effect was observed. Statistical significance was declared at $P<0.05$. DCA, dichloroacetate. (pre dose) = resting sample taken before DCA administration. 3min = 3 minutes from the beginning of the transition. 6min = 6 minutes from the beginning of the transition. 3, 6 and 9min post = 3, 6 and 9 minutes post exhaustion respectively. DCA = Dichloroacetate; SBC = Standard Bicarbonate; Gluc. = Glucose; La. = Lactate; (pre dose) = pre treatment sample.

5.2.2 The effect of DCA on exercise performance

A reduction in blood lactate throughout exercise was not reflected in an increase in time to exhaustion (T_{Lim}) in the DCA trial. T_{Lim} for DCA and placebo trials was 12.60 ± 1.13 min and 14.20 ± 1.53 min respectively (Mean ± SEM. P = 0.10).

5.2.3 $\dot{V}O_2$ responses to 'heavy' intensity exercise

The group mean ± SEM on-transient $\dot{V}O_2$ responses are shown in figure 54. There is no difference between the DCA and placebo trials. This is reflected in the $\dot{V}O_2$ values at baseline (Mean ± SEM. 0.80 ± 0.039 l/min DCA. 0.79 ± 0.032 l/min placebo. P = 0.65. Table 12), minute 3 (Mean ± SEM. 3.08 ± 0.17 l/min DCA. 3.09 ± 0.16 l/min placebo. P = 0.82. Table 12), minute 6 (Mean ± SEM. 3.30 ± 0.16 l/min DCA. 3.30 ± 0.16 l/min placebo. P = 0.79. Table 12) and exhaustion (Mean ± SEM. 3.35 ± 0.15 l/min DCA. 3.34 ± 0.18 l/min placebo. P = 0.62. Table 12).

The fundamental $\dot{V}O_2$ time constant was not significant different between DCA and placebo trials (Mean ± SEM. 31 ± 2.9 s DCA. 31 ± 2.6 s placebo. P = 0.91. Table 12). A mono-exponential function (*Eqn 20*) was fitted to the first 3 minutes of the $\dot{V}O_2$ response excluding the initial 'cardiodynamic' (~25 s) phase. Figures 55 a,b&c show the individual plots for each subject. The residuals associated with each of the plots fluctuate closely and randomly around zero. This indicates that this phase of the response was well described by the mono-exponential function. The fit remained good for the first 3 minutes with a small divergence in the residuals only evident in subjects 3, 4 and 7.

An additional slow component of the $\dot{V}O_2$ response is clearly evident after 3 min in all subjects (fig. 55 a,b&c). The initial rise in this excess component can be estimated by expressing it as the mean of the 6th min minus the mean of the 3rd min. This difference was positive for all subjects. There was no difference between DCA and placebo trials (Mean $\pm$ SEM. 0.22 $\pm$ 0.047 l/min DCA. 0.22 $\pm$ 0.034 l/min placebo. P = 0.96. Table 12). The asymptotic steady-state $\dot{V}O_2$, predicted from the mono-exponential fit through the first 3 min was not significantly different from the average of minute 3 for both groups. This was true for all subjects indicating that the influence of a slow component on the modelled fundamental $\dot{V}O_2$ responses was at most minimal. Therefore, the choice of 3 min was an appropriate time frame for modelling using the mono-exponential fit.

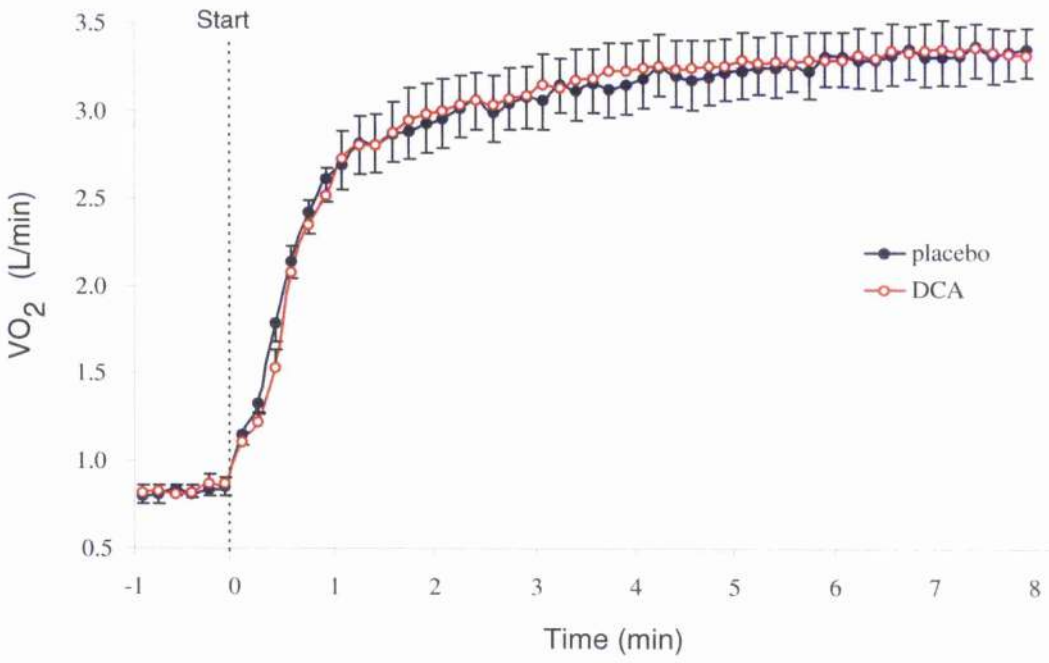


Figure 54 – Data are group mean ± SEM. The on-transient $\dot{V}O_2$ response to heavy intensity exercise. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

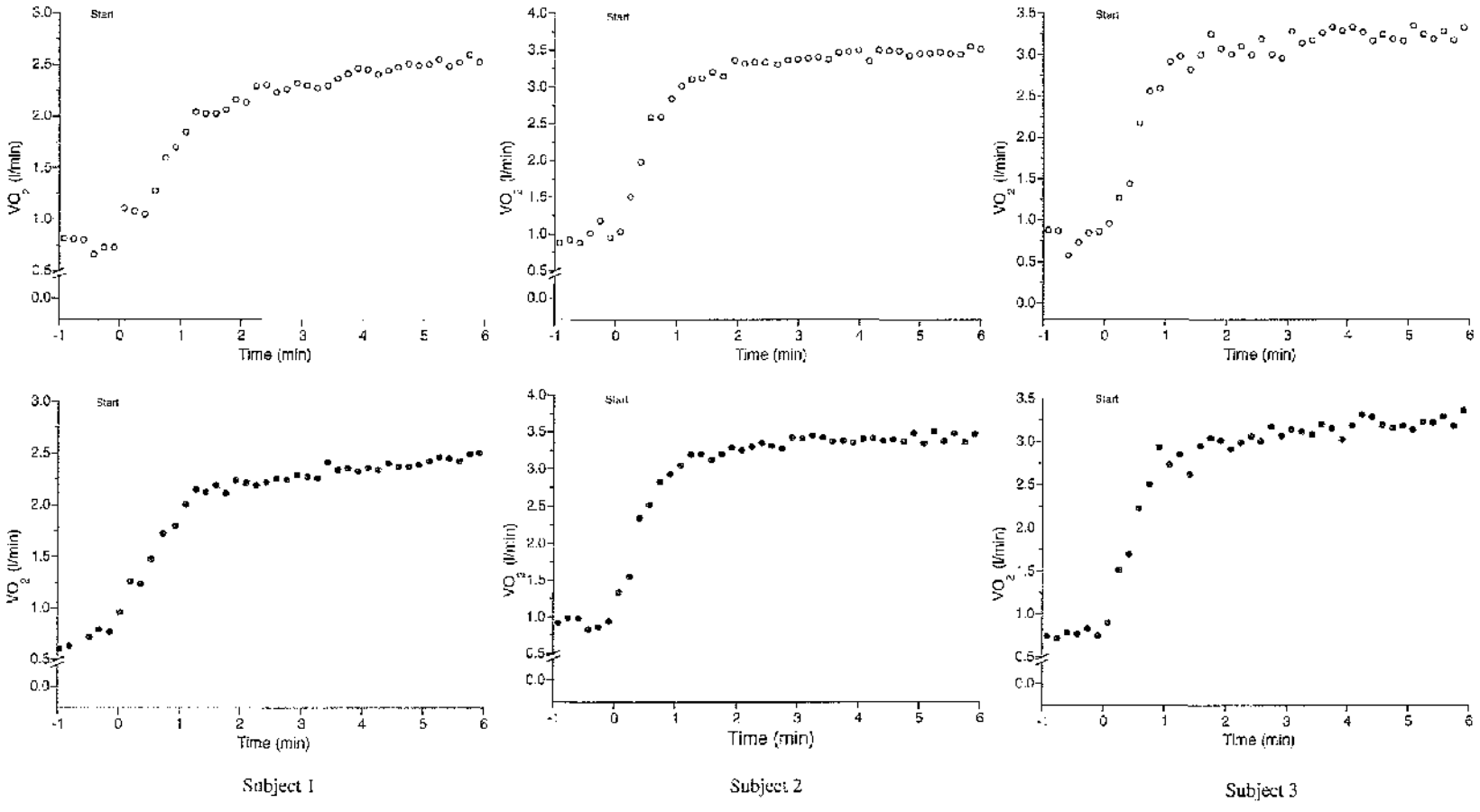


Figure 55a – Exponential fit to the fundamental component of the $\dot{V}O_2$ response to supra-threshold square wave exercise. Individual plots. Subjects 1-3. Open circles = DCA. Closed circles = placebo. The solid line through the data points indicates the mono-exponential fit. The fit began at least 20 s into exercise to exclude the cardiodynamically mediated 'phase 1' response and continued through to 3 min. The dashed continuation of this line shows the fit extrapolated to 6 min. The 'goodness' of fit is illustrated by the fluctuation of the residuals around zero. The smaller these fluctuations are the better the fit is. The vertical dashed line indicates the start of the stepped increase in work rate.

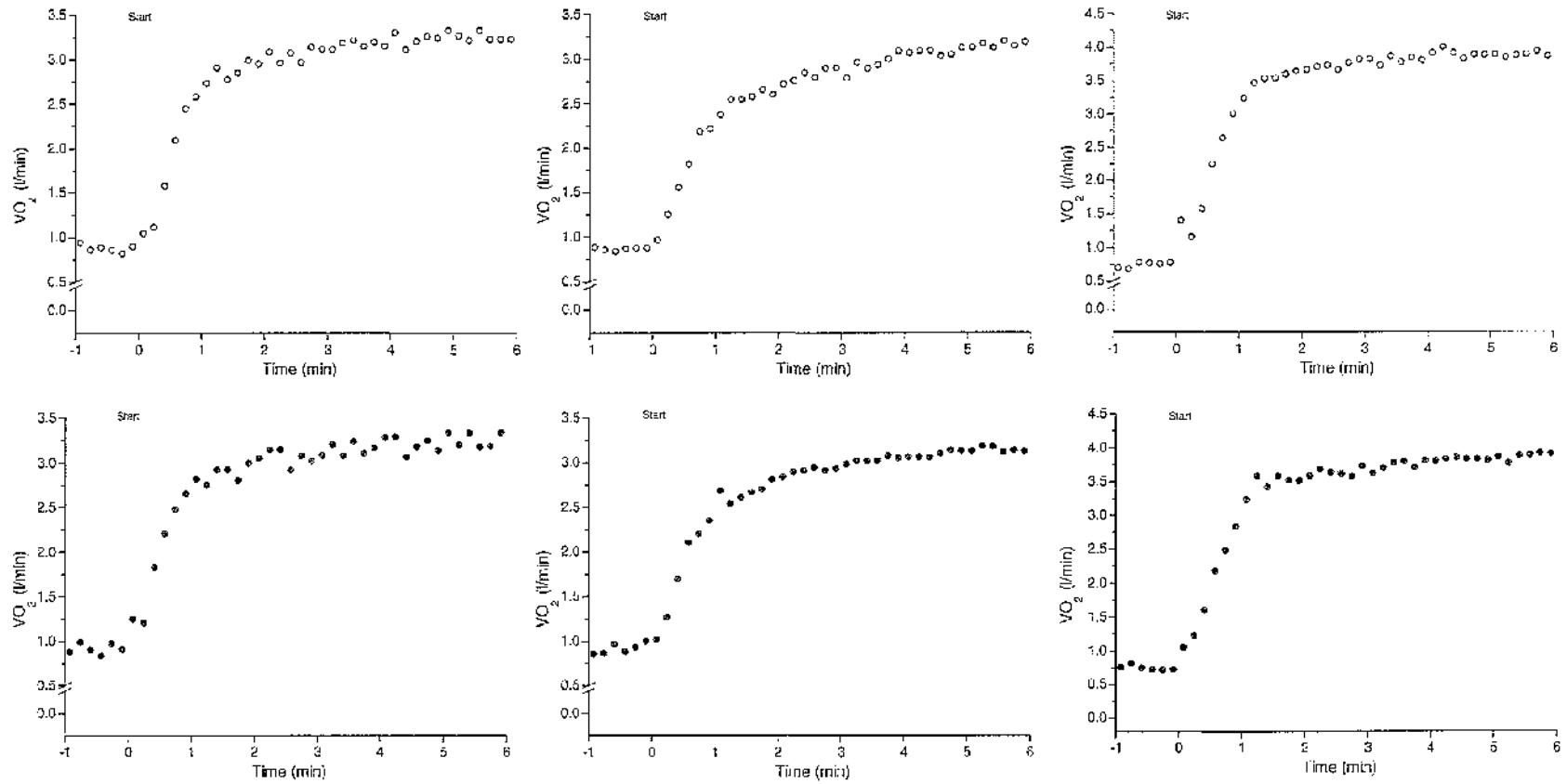
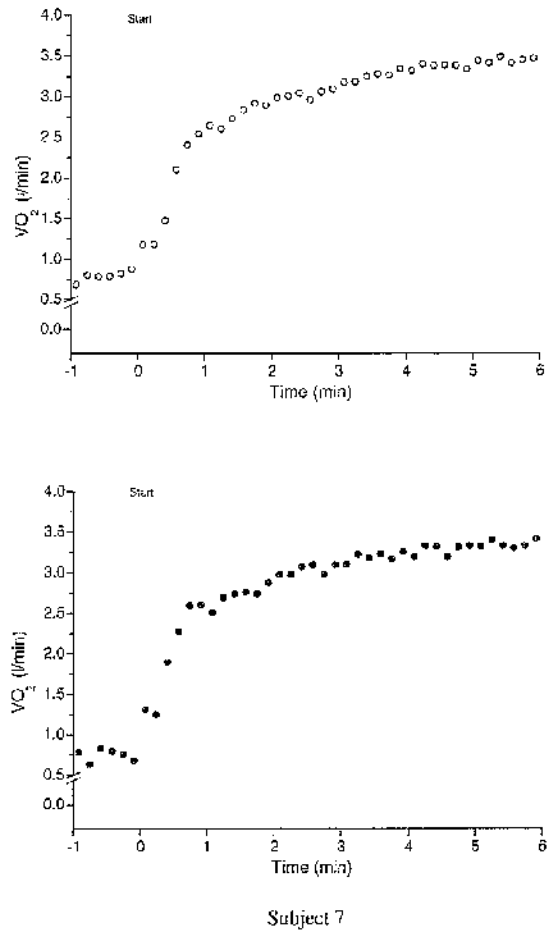


Figure 55b – Exponential fit to the fundamental component of the $\dot{V}O_2$ response to supra-threshold square wave exercise. Individual plots. Subjects 4-6. Open circles = DCA. Closed circles = placebo. The solid line through the data points indicates the mono-exponential fit. The fit began at least 20 s into exercise to exclude the cardiodynamically mediated 'phase 1' response and continued through to 3 min. The dashed continuation of this line shows the fit extrapolated to 6 min. The 'goodness' of fit is illustrated by the fluctuation of the residuals around zero. The smaller these fluctuations are the better the fit is. The vertical dashed line indicates the start of the stepped increase in work rate.



Subject 7

Figure 55c - Exponential fit to the fundamental component of the $\dot{V}O_2$ response to supra-threshold square wave exercise. Individual plot. Subject 7. Open circles = DCA. Closed circles = placebo. The solid line through the data points indicates the mono-exponential fit. The fit began at least 20 s into exercise to exclude the cardiodynamically mediated 'phase I' response and continued through to 3 min. The dashed continuation of this line shows the fit extrapolated to 6 min. The 'goodness' of fit is illustrated by the fluctuation of the residuals around zero. The smaller these fluctuations are the better the fit is. The vertical dashed line indicates the start of the stepped increase in work rate.

Table 12 - $\dot{V}O_2$ responses to 'heavy' intensity exercise

Subject	τ (s)		$VO_{2\,20}$ (l/min)		VO_{2m3} (l/min)		VO_{2m6} (l/min)		VO_{2ex} (l/min)	
	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA
1	34	40	0.62	0.72	2.28	2.31	2.50	2.50	2.52	2.61
2	35	31	0.92	0.92	3.42	3.37	3.48	3.52	3.50	3.48
3	21	20	0.80	0.77	3.11	3.11	3.34	3.32	3.24	3.35
4	29	27	0.87	0.86	3.07	3.11	3.36	3.25	3.31	3.35
5	43	42	0.87	0.87	2.96	2.84	3.14	3.19	3.23	3.22
6	28	28	0.71	0.73	3.67	3.81	3.89	3.87	4.09	3.94
7	29	32	0.76	0.72	3.09	3.02	3.43	3.43	3.47	3.52
Mean	31	31	0.79	0.80	3.09	3.08	3.30	3.30	3.34	3.35
SEM	2.6	2.9	0.032	0.039	0.16	0.17	0.16	0.16	0.18	0.15
P value		0.91		0.65		0.82		0.79		0.62

Subject	VO_2 6-3 (l/min)		VO_{2asy} (l/min)		Δ VO_2/ ΔWK ($ml \cdot min^{-1} \cdot W^{-1}$)		T_{Lim} (min)	
	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA
1	0.20	0.19	2.27	2.31	9.82	9.46	8.10	9.63
2	0.06	0.16	3.36	3.36	9.60	9.76	16.48	16.08
3	0.23	0.21	3.01	3.08	9.44	9.87	17.68	14.28
4	0.29	0.14	3.06	3.06	9.73	9.73	16.62	15.12
5	0.19	0.35	2.97	2.86	9.59	9.13	8.97	9.13
6	0.22	0.07	3.67	3.76	9.55	9.77	17.43	14.07
7	0.34	0.42	2.98	3.05	8.70	9.14	14.18	9.60
Mean	0.22	0.22	3.05	3.07	9.49	9.55	14.20	12.60
SEM	0.034	0.047	0.16	0.17	0.15	0.17	1.53	1.13
P value		0.96		0.41		0.69		0.10

Data are individual values. Group mean ± SEM data shown. * Statistically significant. Statistical analysis was carried out using a paired *t*-test. Statistical significance was declared at P<0.05. 20 = Low load pedalling (20 W). τ = time constant for a standardised exponential fit over the first 3 minutes. Δ $\dot{V}O_2$ / Δ WK = steady state gain projected from exponential fit divided by the delta change in work rate. $\dot{V}O_2$ 6-3 = min 6 $\dot{V}O_2$ - min 3 $\dot{V}O_2$. $\dot{V}O_2$ (ex) = end exercise $\dot{V}O_2$. T_{Lim} = time to exhaustion.

5.2.4 Cardiovascular responses to 'heavy' intensity exercise

Heart rate was significantly elevated at rest by a mean of 9 beats·min^{-1} during the DCA trial (Mean ± SEM. 70 ± 2.3 beats·min^{-1} DCA, 61 ± 1.0 beats·min^{-1} placebo. P = 0.013). This elevation in heart rate was maintained during low load baseline pedalling (Mean ± SEM. 100 ± 3.7 beats·min^{-1} DCA, 90 ± 2.5 beats·min^{-1} placebo. P = 0.012. Table 13). By 3 min into exercise this effect is no longer seen. No differences in heart rate are discernable at 3min, 6min or exhaustion (summary data are shown in table 13). This elevation in baseline heart rate can be clearly seen in figure 56 which shows the group mean ± SEM responses for both trials. Figures 57 shows that when normalised to a zero baseline the delta heart rate requirement is significantly lower in the DCA trial.

From the $\dot{V}O_2$ and heart rate responses it follows that the oxygen pulse was found to be significantly lower at rest during the DCA trial (Mean ± SEM. 6.77 ± 0.44 ml/beat DCA. 8.00 ± 0.58 ml/beat placebo. P = 0.02. Table 13). This reduction was still evident during baseline low load pedalling (Mean ± SEM. 8.09 ± 0.54 ml/beat DCA. 8.84 ± 0.58 ml/beat placebo. P = 0.02. Table 13). No significant difference was found at any other time point (summary data are shown in table 13). The reduction in baseline oxygen pulse can be clearly seen in figure 58 which shows the group mean ± SEM responses for both trials. Figure 59 shows the same data corrected for differences in baseline values. It is evident from this graph that the time course and magnitude of the responses are similar for both trials.

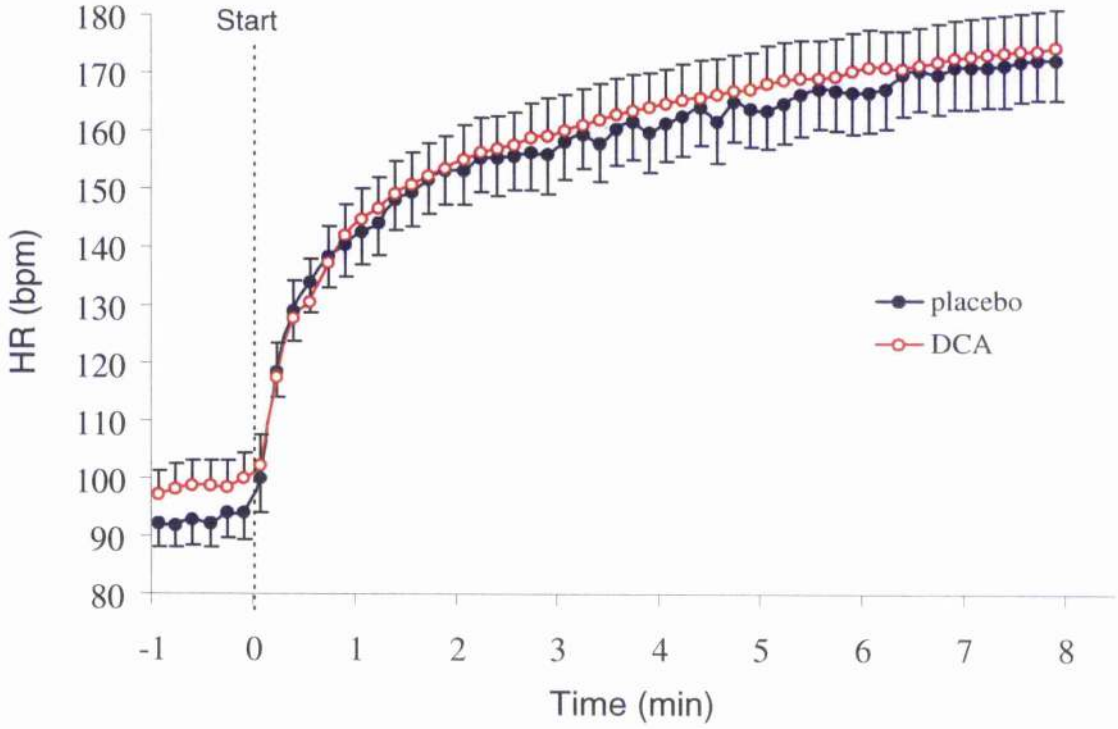


Figure 56 – The heart rate response to heavy intensity exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

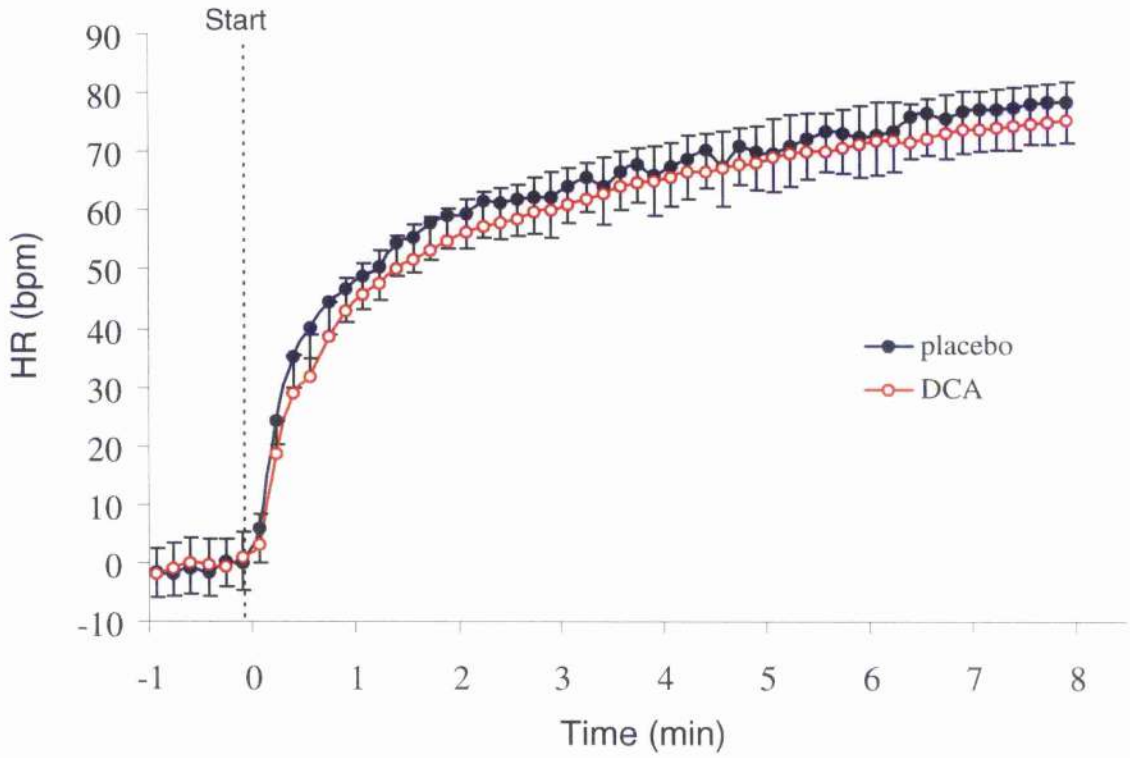


Figure 57 – The heart rate response to heavy intensity exercise, normalised to baseline values. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

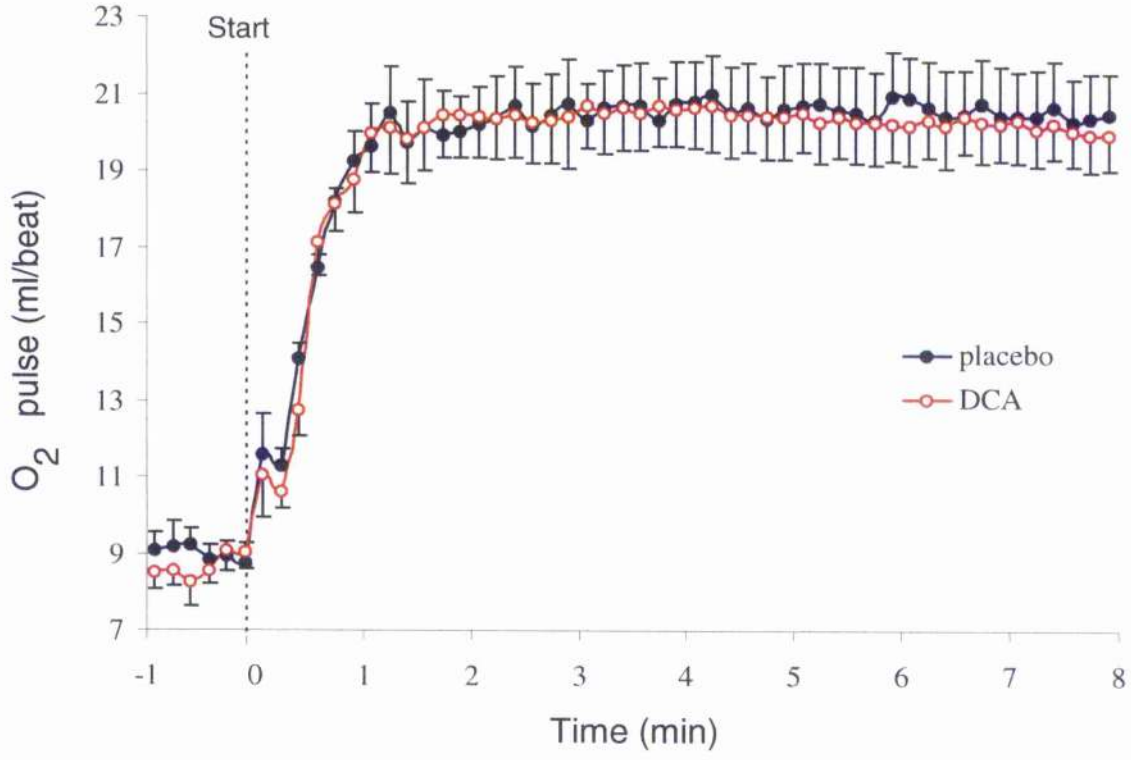


Figure 58 – The oxygen pulse response to heavy intensity exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

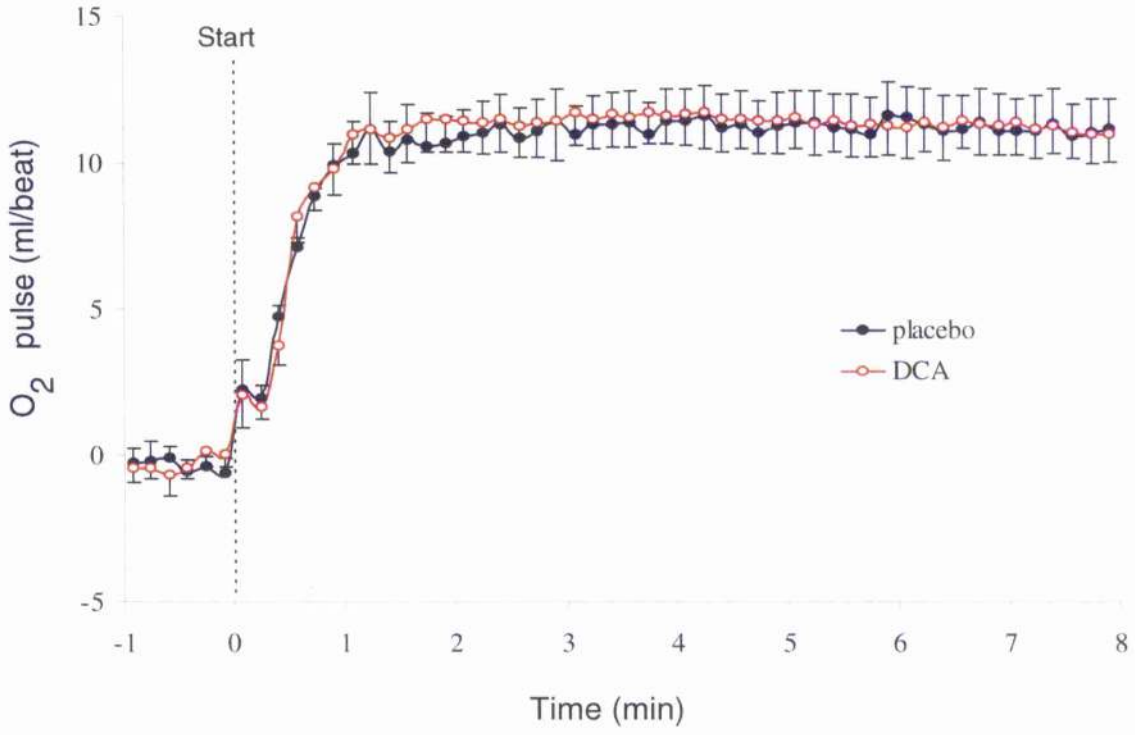


Figure 59 – The oxygen pulse response to heavy intensity exercise, normalised to baseline values. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

Table 13 - Cardiovascular response to 'heavy' intensity exercise

Subject	Hr_{rest} (bpm)		Hr_{20} (bpm)		Hr_{m3} (bpm)		Hr_{m6} (bpm)		Hr_{ex} (bpm)	
	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA
1	-	-	97	107	167	164	182	178	184	181
2	62	66	85	87	132	135	139	143	151	156
3	57	63	79	89	147	147	153	159	166	169
4	64	74	92	100	166	172	178	179	187	186
5	62	78	94	105	166	160	183	176	189	184
6	60	68	89	98	164	165	175	175	188	185
7	-	-	97	114	170	173	178	181	184	182
Mean	61	70*	90	100*	159	159	170	170	178	178
SEM	1.0	2.3	2.5	3.7	5.3	5.2	6.4	5.3	5.5	4.2
P value		0.013		0.0012		0.72		0.81		0.55

Subject	O_2Pulse_{rest} (ml/beat)		O_2Pulse_{20} (ml/beat)		O_2Pulse_{m3} (ml/beat)		O_2Pulse_{m6} (ml/beat)		O_2Pulse_{ex} (ml/beat)	
	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA	Placebo	DCA
1	-	-	6.39	6.73	13.65	14.09	13.74	14.04	13.70	14.42
2	8.49	6.92	10.82	10.57	25.91	24.96	25.04	24.62	23.18	22.31
3	7.77	7.44	10.13	8.65	21.16	21.16	21.83	20.88	19.52	19.82
4	-	-	9.46	8.60	18.49	18.08	18.88	18.16	17.70	18.01
5	6.49	5.49	9.26	8.29	17.83	17.75	17.16	18.13	17.09	17.50
6	9.22	7.22	7.98	7.45	22.38	23.09	22.23	22.11	21.76	21.30
7	-	-	7.84	6.32	18.18	17.46	19.27	18.95	18.86	19.34
Mean	8.00	6.77*	8.84	8.09*	19.66	19.51	19.73	19.56	18.83	18.96
SEM	0.58	0.44	0.58	0.54	1.48	1.41	1.40	1.28	1.18	0.99
P value		0.02		0.02		0.55		0.49		0.58

Data are individual values. Group mean ± SEM data shown. * Statistically significant between DCA and placebo trials. Statistical analysis was carried out using a paired *t*-test. Statistical significance was declared at $P<0.05$. 20 = Low load pedalling (20W). m3 = minute 3. m6 = minute 6. ex = exhaustion.

5.2.5 Muscle oxygen saturation during 'heavy' intensity exercise

Figure 60 shows the group mean ± SEM deoxyhaemoglobin plots for both trials. The plots are well matched at baseline and throughout exercise. The initial drop in Hb seen at the beginning of the step change in work rate in chapter 4 is evident again in these results (refer to section 4.2.4). After an initial rapid response the plots show a slower rise in the Hb response developing approximately 1-2 min into exercise. This is much early than the slow component of the $\dot{V}O_2$ response which only became evident at approximately 3 min into exercise.

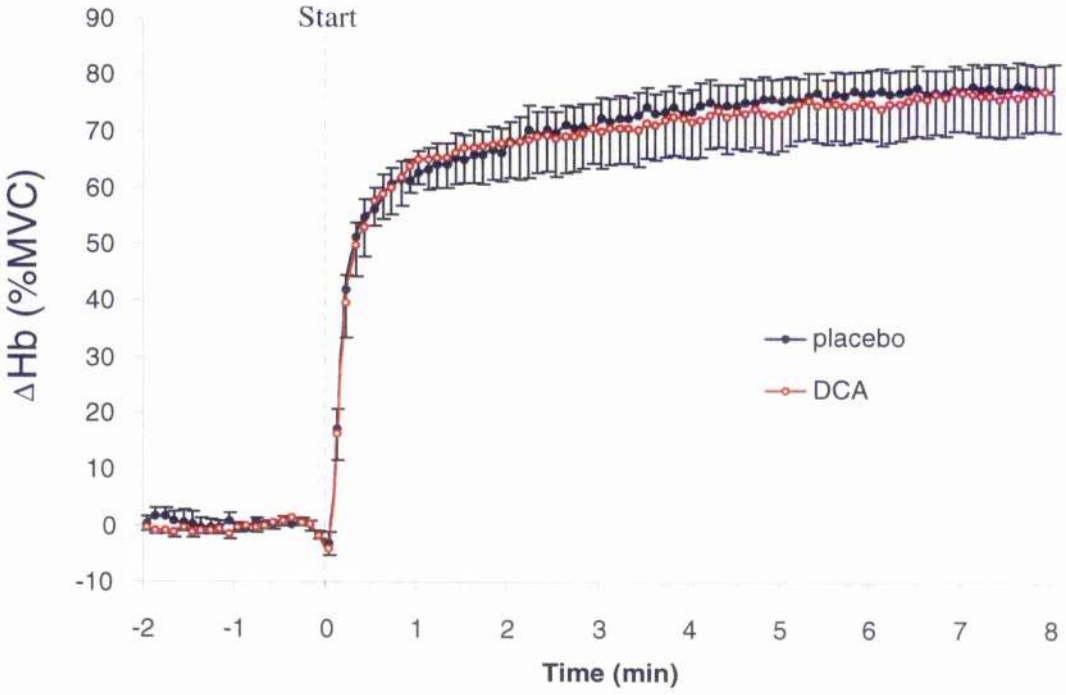


Figure 60 - Delta deoxyhaemoglobin responses to heavy intensity exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. Dashed line indicates the start of the step increase in work rate from a 20 W baseline.

5.2.6 Ratings of perceived exertion and dyspnoea during 'heavy' intensity exercise

No significant difference was found in rating of perceived exertion or rating of dyspnoea between the two trials at any time point. Figure 61 shows the group mean ± SEM values for ratings of perceived exertion. Figure 62 shows the group mean ± SEM values for ratings of dyspnoea.

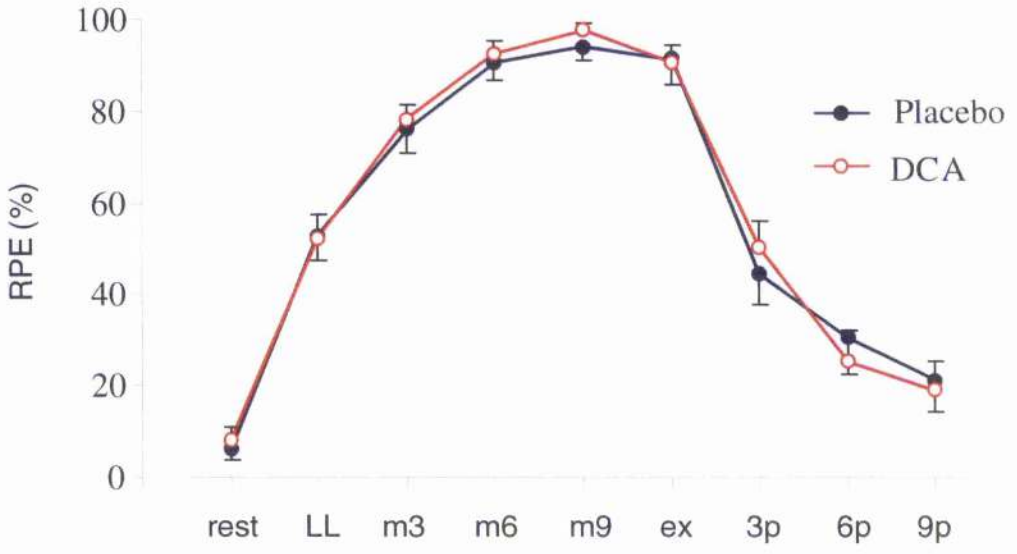


Figure 61- Ratings of perceived exertion during heavy intensity exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. LL = low load pedalling (20W). m3, m6, m9 = minutes 3,6, & 9 respectively. Ex = exhaustion. 3p, 6p, 9p = minutes 3, 6, & 9 post respectively.

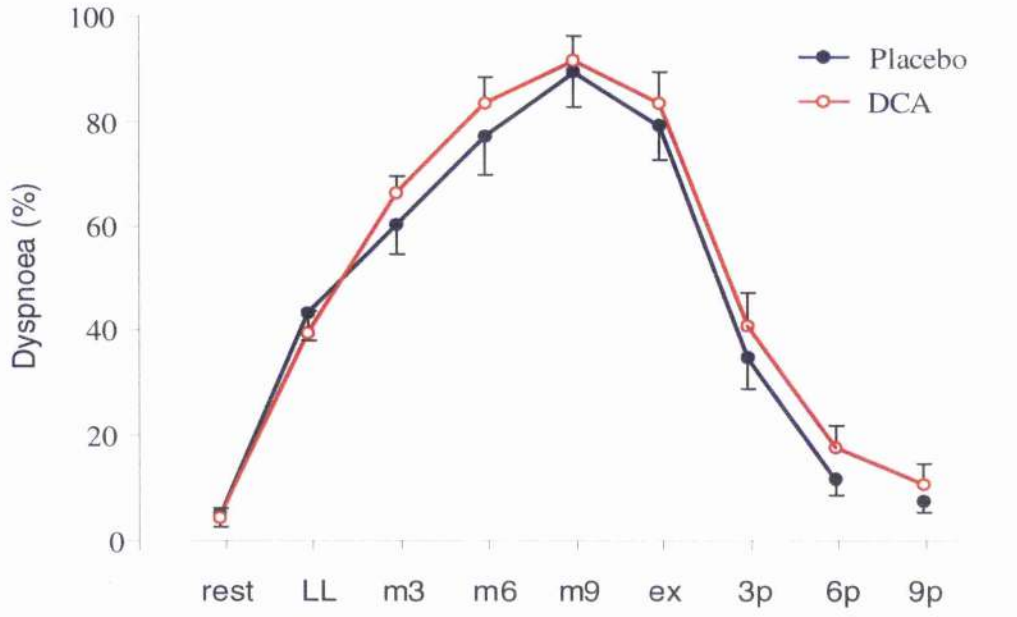


Figure 62 - Ratings of dyspnoea during heavy intensity exercise. Data are group mean ± SEM. Open circles = DCA. Closed circles = placebo. LL = low load pedalling (20W). m3, m6, m9 = minutes 3,6, & 9 respectively. Ex = exhaustion. 3p, 6p, 9p = minutes 3, 6, & 9 post respectively.

5.3.1 The effect of DCA on the fundamental $\dot{V}O_2$ time constant and gain

The initial phase of the $\dot{V}O_2$ response was well fitted by a mono-exponential response as previously described (Paterson and Whipp, 1992; Ozyener *et al.*, 2001) and the tau for this response (~30s) was similar to that expected for sub-threshold exercise (Whipp, 1971; Whipp and Wasserman, 1972; Davies *et al.*, 1972; Bakker *et al.*, 1980; Whipp and Ward, 1990; Whipp *et al.*, 1982; Hughson and Morrissey 1982). No difference was found however between the time constant of the response for DCA and placebo trials. Indeed with the exception of subject 1 the values for tau were within 4s of each other for all subjects. This finding is similar to that of Rossiter *et al* (2003) who reported no difference in $\dot{V}O_2$ on-kinetics during single leg knee extensor exercise before and after DCA administration. Similar results have been reported by Bangsbo *et al* (2002) and Savasi *et al* (2002) for higher intensity exercise in humans and Grassi *et al* (2002) for electrically induced exercise in dog gracilis muscle. However, where the results of these studies differ from that of Rossiter *et al* (2003) is in the amplitude of the fundamental $\dot{V}O_2$ and PCr responses, and level of reduction in blood [lactate]. Rossiter *et al* (2003) found that while the tau for the fundamental $\dot{V}O_2$ and PCr responses was unaltered the steady-state gain of the responses was reduced, coupled with this was a reduced blood [lactate] and an increase in pH throughout exercise. In contrast the studies by Bangsbo *et al* (2002), Savasi *et al* (2002) and Grassi *et al* (2002) reported no difference between the PCr, $\dot{V}O_2$ and lactate responses between the DCA and placebo trials.

The data from Rossiter *et al* (2003) agrees with previous studies showing a reduced PCr breakdown after DCA administration (Timmons *et al.*, 1998; Howlett *et al.*, 1999; Parolin *et al.*, 2000). The reasons for the discrepancy between these studies and those of Bangsbo *et al* (2002), Savasi *et al* (2002) and Grassi *et al* (2002), may be related to the intensity of the exercise undertaken. The studies showing no difference in PCr breakdown were undertaken at a much higher intensity than those that do. Why this is the case is uncertain. The exercise intensity for this study was chosen to allow time for the slow component to develop, and to elicit fatigue in under ~20 min. Therefore comparison with the studies of Rossiter *et al* (2003), Timmons *et al* (1998), Howlett *et al* (1999) and Parolin *et al* (2000) are more apt for the initial phase of the response. However none of the aforementioned studies have continued the work rate protocol until exhaustion and only the study of Rossiter *et al* (2003) described the $\dot{V}O_2$ kinetic response.

The reduction in the fundamental $\dot{V}O_2$ gain demonstrated in the study by Rossiter *et al* (2003) and inferred by Timmons *et al* (1998), Howlett *et al* (1999) and Parolin *et al* (2000) is not evident in the $\dot{V}O_2$ responses from this study. The results of Rossiter *et al* (2003) agree with those of Greenhaff *et al* (2002) who showed that the anaerobic component of ATP production was reduced after DCA administration. The mechanism behind this apparent reduction in ATP requirement are proposed by Rossiter *et al* (2003) to include 1) a shift to a greater contribution from muscle fibres with a lower '$\dot{V}O_2$ gain'; 2) a reduction in intramuscular pH (in either strongly acid-producing muscle fibres or in the muscle as a whole); 3) a greater reliance on oxidative carbohydrate metabolism; and 4) a greater mechanical efficiency of performing the task.

The reason for the difference between the results from this study and those mentioned are not clear. However Rossiter *et al* (2003), when discussing greater mechanical efficiency, mentioned that all of their subjects spontaneously reported that exercise felt "easier" after DCA administration. In contrast no significant difference was found in this study between ratings of either RPE or dyspnoea. Indeed many of the subjects reported that the exercise felt "harder" after DCA administration, despite their remaining blinded to the experimental condition. This may suggest that the results could be specific to the mode of exercise. Differences between knee extensor exercise and upright cycling have been noted previously (Rossiter *et al.*, 2001 vs. Burnley *et al.*, 2000), however no reason for these differences were given. In the Rossiter *et al* (2003) study, the exercise was carried out in a face down supine position. Koga *et al* (1999) demonstrated a reduced fundamental gain with no change in the $\dot{V}O_2$ time constant when heavy exercise was performed in the supine position. These results were thought to be as a consequence of a reduced perfusion pressure due to attenuated O_2 delivery. While it is not expected that DCA causes a reduction in O_2 delivery, the increase in heart rate upon DCA administration described in this study suggests that the haemodynamic properties of DCA are yet to be fully understood. The results of Koga *et al* (1999) demonstrate that the time constant and gain of the fundamental $\dot{V}O_2$ response can be dissociated. Furthermore they show that these parameters are sensitive to the mode of exercise employed.

5.3.2 The effect of DCA on the slow component $\dot{V}O_2$ response

Similar to the initial phase of the exercise response, DCA had no effect on the subsequent slow component response. Indeed the $\dot{V}O_2$ response for all subjects was evidently similar in all subjects for both trials. The only comparable study for this work intensity is that of Rossiter *et al* (2003). Once more the results of the two studies differ. Rossiter *et al* (2003) observed, on average, a ~28% reduction in the $\dot{V}O_2$ slow component between the DCA and placebo trial and this was accompanied by a 32% reduction in end-exercise blood [Lactate]. A 12% reduction in blood lactate was observed in end-exercise blood [Lactate] in this study with no reduction in the slow component. Therefore an acid stress mediated reduction in the magnitude of the slow component is not supported by these results. The reason for this disparity between the studies is once again unclear. However Rossiter *et al* (2003) make the interesting observation that, when expressed as a percentage of the fundamental $\dot{V}O_2$ gain, there is no difference between magnitude of the slow component between DCA and placebo trials. Following this hypothesis, as there is no difference in the fundamental response between the two trial conditions in this study, a difference in the slow component response is not expected. Therefore the data set from this study would fit a theory that the fundamental response plays a role in determining the magnitude of the slow component response. However this experimental design presents a poor interrogation of this hypothesis and, as stated by Rossiter *et al* (2003), there is currently little evidence available to support this theory. Indeed, Jones *et al* (2002) demonstrated a reduction of the fundamental component of the $\dot{V}O_2$ response to heavy exercise with creatine supplementation and this reduction was not accompanied by a concomitant reduction of the slow component response.

While the reasons for the disparity between the Rossiter *et al* (2003) study and this one are unclear it is worth noting that the metabolic demand associated with the two forms of exercise is markedly different. For example, the peak exercise blood [lactate] in the Rossiter *et al* (2003) study was 2.7 ± 1.8 (mmol/l) compared with 13.49 ± 3.80 (mmol/l) in this study. The Δ $\dot{V}O_{2ss}$ was 0.84 ± 0.21 (l/min) in the Rossiter *et al* (2003) compared with 2.27 ± 0.4 (l/min) in this study. Therefore while the acid stress was reduced in Rossiter *et al* (2003) study it was reduced from an already low background. The subsequent reduction in the slow component, assuming cause and effect, was of the magnitude 0.07 (l/min). Therefore, it may be that the greater metabolic demand of the exercise in this study precludes/masks any appreciable effect of DCA on the O_2 kinetic response. This could possibly be due to differences in proportional activation of PDH. PDH has been shown to be activated fully shortly into higher intensity exercise (Putman, 1995). Or perhaps the greater variance in the oxygen uptake measures with this protocol did not allow for discrimination of small changes in $\dot{V}O_2$. Interestingly, if the magnitude of the reduction in the slow component from the Rossiter *et al* (2003) study is factored by the observed difference in the Δ $\dot{V}O_{2\,ss}$ values between that study and this (i.e. ~x3), the resulting change of 0.21 (l/min) lies within 1SD of the mean $\dot{V}O_2$ values reported for both conditions in this study. This further suggests that any appreciable change in $\dot{V}O_2$ may be to small to detect with this experimental design. Howlett and Hogan (2003), upon demonstrating a speeding of the fall in Po_2 with DCA at the onset of contraction in isolated *Xenopus* single muscle fibres, questioned whether the differences in oxidative metabolism shown in their preparation were large enough to be discernable in whole muscle preparations.

The results from this study therefore do not support a role for PDH in either a significant speeding of the fundamental component of the $\dot{V}O_2$ response or an attenuation of the slow component response to dynamic exercise in the 'heavy' exercise domain.

5.3.3 The effect of DCA on muscle oxygen saturation

The deoxyhaemoglobin responses show some marked yet inconsistent differences between DCA and placebo trials. As discussed previously (see section 5.2.5) the acid shift evoked by the H+ ion associated with lactate facilitate the unloading of O_2, illustrated by a right shift of the oxyhaemoglobin dissociation curve. Therefore a marked reduction in blood [lactate] through PDH activation in the working muscle may attenuate the rise in muscle deoxygenation. This effect is not apparent in this study.

The profile of the deoxyhaemoglobin plot is very similar to that of $\dot{V}O_2$ in that after an initial rapid response a second slower response is evident 1-2 min into exercise. Poole *et al* (1991) and Rossiter *et al* (2002) have shown that ~70% of the $\dot{V}O_2$ slow component originates from within the exercising muscle mass and this is reflected by the increase in the deoxyhaemogloin plots here. Interestingly, figures 55a&b show the first 3 min of the $\dot{V}O_2$ response are fit well by an exponential response, with divergence in the residuals only evident in subject 1 (after ~2 min); however it is clear from the deoxyhaemoglobin plots that the 'slow component' of the response is manifest within 3 min of exercise onset. A previous study by Belardinelli *et al* (1995) showed an increase in both $\dot{V}O_2$ and muscle deoxygenation after 3 min of constant work exercise, however the data from their study did not allow discrimination of an exact point of change of the two responses. While an attempt has not been made in this study to discern an exact

point of increase in the 'slow component' of the deoxyhaemoglobin plot, a clear divergence can be seen visually to occur within the first 2 min of exercise. The greater data density in this study compared to the Belardinelli *et al* (1995) study allowed for better discrimination of the initial signs of divergence in the deoxyhaemoglobin plot. Therefore it appears that the slow component of the response may be evident in the muscle significantly earlier than it is evident in pulmonary $\dot{V}O_2$. A possible reason for this is that the relatively small region under interrogation may not reflect the energetics of the combined exercising musculature. Therefore the slow component may develop more quickly in one region of the muscle in relation to the other and this may be borne out in pulmonary $\dot{V}O_2$ as a mean response. This heterogenic $\dot{Q}musO_2$ response within a muscle group has been reported by Quaresima *et al* (2001). Indeed Van Beekvelt *et al* (2001) reported that the Fick method of determining whole muscle O_2 uptake did not correlate with the regional assessment from NIRS. This disparity between local muscular oxygen uptake and whole muscle/whole body oxygen uptake could be a consequence of local perfusion-metabolism mismatching (Grassi, 2001) or, as discussed by Whipp *et al* (2002), individual oxygen uptake responses for each muscle fibre. Either of these two would lead to a mean response that differed from a local muscular response. The extent and significance of this difference requires further investigation. However these results present interesting questions on modelling the phase II-phase III $\dot{V}O_2$ transition and its relevance to the onset of the slow component response.

5.3.4 The effect of DCA on heart rate

In agreement with the first two studies, heart rate was significantly increased at rest and during unloaded pedalling during the DCA trial. However this marked increase was not continued throughout exercise. Group averages across minute 3, minute 6 and at exhaustion showed no variance between trials. Delta heart rate responses were once again consistently lower on the DCA trial, illustrating a lower heart rate recruitment. This suggests the effects of DCA on heart rate are not additive throughout the period of exercise and/or the mechanism/s by which heart rate is increased by DCA are functionally no different to those increasing heart rate during the placebo trial. As with previous chapters this subject will be covered in more detail during the general discussion. Oxygen pulse is reduced as a result of the elevated heart rate response at rest and during low load pedalling.

5.3.5 Exercise performance and fatigue

Of the main factors thought to contribute to muscle fatigue during exercise such as [Pi], pH, alterations in Ca^{2+} release and sensitivity and muscle glycogen content; DCA has been shown to improve the [Pi] and pH responses and elicit a muscle glycogen sparring effect (Timmons *et al.*, 1996; Grassi *et al.*, 2002; Schneider *et al.*, 1981; Durkot *et al.*, 1995; Rossiter *et al.*, 2003). Therefore there appears to be a role for DCA in improving muscle function and reducing fatigue.

Timmons *et al* (1996) demonstrated that DCA reduced muscle fatigue by ~35% during 20 min of ischaemic contraction in a canine model. Similarly Grassi *et al* (2002) found that the fatigue index in electrically stimulated dog gracilis muscle was improved by

~8% after DCA infusion during a 4 min electrically stimulated isometric tetanic contraction, corresponding to ~60-70% of peak $\dot{V}O_2$. Schneider *et al* (1981) found rats dosed with DCA to swim an additional 99 s before exhaustion. Durkot *et al* (1995) found improved run times in DCA treated rats when compared to controls (169 min vs. 101 min). In both the Durkot *et al* (1995) and Schneider *et al* (1981) studies there was a glycogen sparing effect in the DCA trial.

Of the studies that include humans exercising until exhaustion (Ludvik *et al.*, 1993; Carraro *et al.*, 1989; Roberts *et al.*, 1997), only Ludvik *et al* (1993) report an improved performance. Each of these studies employed an incremental exercise protocol to exhaustion. No previous studies have looked at an exercise trial in humans to determine whether DCA has a ergogenic effect on performance.

No significant difference was seen between trials in this study. This suggests that any improvement in muscle function at the onset of exercise was not sufficient to delay the onset of fatigue in this study. Therefore DCA does not appear to improve muscular function during prolonged 'heavy' intensity exercise. This will be discussed further in the general discussion.

Chapter 6

General Discussion

Chapter 6

6.0 General Discussion

The results from these studies do not support those from previous studies (Howlett *et al.*, 1999; Timmons *et al.*, 1996, Timmons *et al.*, 1998) whose findings have advocated a role for the Pyruvate Dehydrogense complex in the determination of the rate of oxygen uptake at the onset of dynamic exercise. However, they are in agreement with the more recent investigations (Bangsbo *et al.*, 2002; Grassi *et al.*, 2002; Rossiter *et al.*, 2003; Jones *et al.*, 2003; Koppo *et al.*, 2004) which have demonstrated that activation of the pyruvate dehydrogenase complex through dichloroacetate administration has no effect on oxygen uptake kinetics.

These latter findings, coupled with the results from chapters 2 and 3 would suggest that there is no apparent 'functional stenosis' at the level of the PDH complex controlling the $\dot{V}O_2$ response to exercise. However, evidence has been presented by Howlett and Hogan (2003) that suggests that a degree of rate limitation may lie, at least in part, at the level of the PDH complex. Howlett and Hogan (2003) demonstrated an accelerated decrease in PO_2 in isolated single frog muscle fibres with DCA administration. This finding agrees with the studies which have advocated the PDH complex as a 'major determinant' of the oxygen deficit through evidence of a reduced level of substrate phosphorylation upon DCA administration (Howlett *et al.*, 1999; Timmons *et al.*, 1996; Timmons *et al.*, 1998). Why therefore has no discernable effect on the O_2 deficit been evident in those studies which have directly measured pulmonary gas exchange? As discussed previously (section 4.2.2), it is likely that the magnitude of the response in the Howlett & Hogan study (~2 s) is too small to be appreciable *in vivo*. This observation was made by the authors themselves. Likewise, Rossiter *et al* (2003) made the

suggestion that, because aerobic ATP production is more efficient than anaerobic ATP production, only a small increase in $\dot{V}O_2$ would be required to compensate for a relatively large reduction in energy provision through nonoxidative pathways, and that this increase in $\dot{V}O_2$ might therefore be too small to detect. Furthermore, Jones *et al* (2003) suggest that the principal effects of DCA on muscle metabolism occur early following the onset of contraction (i.e. in the first 10-20 s) when muscle $\dot{V}O_2$ is low (Behnke *et al.*, 2002; Hogan, 2001) and therefore little influence on $\dot{V}O_2$ kinetics would be expected i.e. much of the information would be incorporated in the 'cardiodynamically' mediated phase I of the pulmonary $\dot{V}O_2$ response. This is in agreement with the findings of Roberts *et al* (2002) who reported that the 'acetyl group deficit', described by Greenhaff *et al* (2002), exists only during the first 10-20 s of exercise and that after this period acetyl groups accumulate in the muscle (Howlett *et al.*, 1999; Savasi *et al.*, 2002). This suggests that after this initial phase, PDH activation, and the availability of acetyl groups is unlikely to be limiting oxidative phosphorlyation. Therefore, activation of PDH by DCA administration may increase the rate of oxygen consumption at the onset of exercise, however it is likely that this effect is appreciably small that it cannot be determined through the measurement of whole body $\dot{V}O_2$. The functional significance of this increase is therefore minimal.

6.1 The accuracy of the pulmonary $\dot{V}O_2$ responses in reflecting a DCA induced muscle metabolic response

As described in section 2.2.8, the breath-by-breath noise associated with gas exchange analysis determines the sensitivity of the kinetic modelling (Lamarra *et al.*, 1987; Rossiter *et al.*, 2002). Every attempt was made in this study to minimise the signal-to-noise ratio by, averaging repeat transitions (where possible), maximising the sub-threshold delta $\dot{V}O_2$, recruiting large muscle groups, excluding breaths outwith 4 standard deviations of the mean response (Lamarra *et al.*, 1987), and familiarising the subjects thoroughly with all protocols and procedures. As a result the 95% confidence intervals for the fundamental kinetic response in these studies averaged 0.15 s and 0.13 s for the sub and supra threshold kinetic analysis respectively. This is consistent with the values reported by Rossiter *et al* (2003), Jones *et al* (2003) and Koppo *et al* (2004). Therefore an appreciable change in the phase II $\dot{V}O_2$ kinetics i.e. greater than 2 s, would be realised by the analysis in this study. Rossiter *et al* (2003) calculated a predicted change in τ of ~3 s for the ~2 mmol/l difference in [PCr] measured in their study (on the basis of no change in the fundamental $\dot{V}O_2$ gain as originally hypothesised). This lay outside the average 95% confidence intervals of $< \pm 2$ s in their study and similarly lies outside the limits reported for the studies in chapters 3 and 4. Therefore it can be said that the study designs afforded adequate discriminability for a significant difference in $\dot{V}O_2$ kinetics to be realised.

6.2 Exercise intensity dependent responses to Dichloroacetate administration.

Much of the discrepancy between the results of DCA studies is likely due to the intensity of the exercise employed. PDH activation has been shown to be highly dependent on work rate (Constantin Teodosiu *et al.*, 1991; Howlett *et al.*, 1998). This is likely to be a primary contributing factor to the differences in muscle metabolic responses observed between studies (Howlett *et al.*, 1999; Timmons *et al.*, 1996; Timmons *et al.*, 1998; Savai *et al.*, 2002; Bangsbo *et al.*, 2002; Howlett *et al.*, 1999). Studies undertaken in 'very hard' or 'severe' exercise domains (Savai *et al.*, 2002; Bangsbo *et al.*, 2002; Howlett *et al.*, 1999) have not reported the effects of DCA on muscle metabolism described for exercise at a lower intensity (Timmons *et al.*, 1998). The 'acetyl group deficit' as described by (Greenhaff *et al.*, 2002) is suggested by Timmons *et al* (1998) to be the major determinant of oxidative phosphorylation during the transition from a lower to higher level of muscular work. However, Roberts *et al* (2002) have suggested that acetyl group availability is likely to limit oxidative metabolism at low to moderate exercise intensities (<65% $\dot{V}O_2$max) and that PDC activation is likely to be sufficiently rapid at high exercise intensities (>90% $\dot{V}O_2$max). Therefore, the intensity of exercise under study is likely to be a major determinant of any expected change in metabolic response post DCA administration.

6.3 The effect of DCA on muscle fatigue and exercise performance

Alterations in the 'fatigue status' or 'fatigue index' may be an important mechanism by which DCA would improve the overall energy status of the exercising muscle (Rossiter *et al.*, 2003). Factors that are thought to contribute to muscle fatigue include alterations in [Ca^{2+}] release and sensitivity, [Pi] and intracellular pH (Allen, 2001; Westerblad, 2002). Rossiter *et al* (2003) have shown improvements in both the [Pi] and intracellular pH responses in response to DCA administration i.e. a decrease in pH elevation on exercise and a decrease in the magnitude of the [Pi] response. Furthermore, an improved muscle performance has been reported in the studies of Grassi *et al* (2002), Roberts *et al* (2002) and Timmons *et al* (1996). Grassi *et al* (2002), using an isolated dog muscle preparation, found that in response to electrical stimulation the fatigue index was improved by ~8% in the DCA condition. This was manifest as a better maintenance of force production at the same QO_2 rather than a reduced QO_2. Jones *et al* (2003) suggested that any positive effect of DCA on muscle energy state in the immediate transition to exercise would be hypothesised to reduce muscle fatigue and enhance performance later in exercise. The supra-threshold exercise in this study is the first reporting the effects of DCA on performance of dynamic exercise to exhaustion at a work intensity that required the subjects to tolerate elevated levels of blood lactate for a sustained period. That no significant difference was seen between trials suggests that any improvement in muscle energy state during the early response of this exercise protocol did not influence performance during the latter stages. Therefore these results do not agree with the argument of Jones *et al* (2003). Furthermore they indicate that none of the reported benefits to muscle function described by previous studies were significant enough to be realised by an improvement in exercise performance for the

type of exercise employed here. The discrepancy between this study and those studies that have shown improvements is muscle performance may relate to the exercise conditions. Many of the studies demonstrating an improved muscle performance employed exercise under ischaemic (Roberts *et al.*, 2002; Timmons *et al.*, 1996; Timmons *et al.*, 1998), or hypoxic (Parolin *et al.*, 2000) conditions. Whereas those studies that maintained an intact circulation (Howlett *et al.*, 1999; Bangsbo *et al.*, 2002; Grassi *et al.*, 2002; Savasi *et al.*, 2002; Jones *et al.*, 2003) report no effect on muscle metabolism and/or $\dot{V}O_2$ kinetics. That DCA had no appreciable effect on exercise performance for the subject group in this study does not mean that further investigation in this area is not warranted. In diseases such as heart failure, exercise capacity is reduced. Reduced cardiac function and in some cases a thickening of the alveolar-capillary membrane, cause under-perfusion and poor oxygen delivery to the working muscle. The subsequent increase in glycolysis causes production of lactate and associated muscle fatigue (Spurway, 1992). Therefore the haemodynamic effects and the lactate lowering properties of DCA may make a difference to exercise performance in patient groups where it may have a significant impact on patient quality of life.

6.4 The effect of DCA on the Oxygen Deficit

Greenhaff *et al* (2002), Timmons *et al* (1998) and Howlett *et al* (1999) have all shown that the anaerobic component of ATP production is reduced during the first few minutes of exercise after DCA administration, indicated by a reduced PCr breakdown and a decrease blood [Lactate]. However, in these studies neither muscle nor pulmonary $\dot{V}O_2$ was measured and therefore assumptions were made that the reduction in substrate phosphorylation reflected an increased mitochondrial ATP production. The results from chapter 4 are in agreement with that from other authors (Koppo *et al.*, 2004; Jones *et al.*,

2004, Rossiter *et al.*, 2003), clearly demonstrating that this is not the case when pulmonary $\dot{V}O_2$ is measured directly. Jones *et al* (2003) suggest that near maximal acetylation of the carnitine pool promotes a shift towards a relatively greater oxidation of carbohydrate over fat. Increasing the amount of ATP resynthesised for a given $\dot{V}O_2$. The enhancement of metabolic efficiency though this mechanism might partly explain the reduction in substrate level phosphorylation observed following DCA infusion (Roberts *et al.*, 2002). However, it should be considered that the effects of PDC activation on muscle $\dot{V}O_2$ might be so small, or might occur so early in the transition to exercise, that they cannot be accurately measured (Howlett and Hogan, 2002; Jones *et al.*, 2004). For example, Rossiter *et al* (2003) make the observation that because aerobic ATP production is more efficient than anaerobic ATP production, only a small increase in $\dot{V}O_2$ would be required to compensate for a relatively large reduction in energy provision through nonoxidative pathways. Furthermore, it has been proposed that the efficiency of exercise might be improved with DCA because the greater flux of acetyl groups promotes increased carbohydrate oxidation over fat oxidation with a corresponding reduction in the O_2 cost of exercise (i.e. increased P-to-O ratio); that might partially mask any increase in $\dot{V}O_2$ resulting from DCA infusion (Grassi *et al.*, 2002; Roberts *et al.*, 2002). However, the ^{31}P magnetic resonance spectroscopy study of Rossiter *et al* (2003) indicates that DCA might improve muscle efficiency by reducing the rate of ATP turnover (as estimated by d[PCr]/dt) for the same work rate. The reduction in substrate –level phosphorylation with DCA reported previously (Howlett *et al.*, 1999; Timmons *et al.*, 1998, Timmons *et al.*, 1996) might therefore reflect the requirement for a lower ATP turnover and need not be explained by any effect on the $\dot{V}O_2$ kinetic response. Therefore, the reduction in the O_2def originally described by Timmons *et al* (1998) could be considered to reflect a reduced amplitude

of the fundamental response and not a decrease in the $\dot{V}O_2$ time constant. Suggesting that the availability of TCA intermediates plays no appreciable role in the determining $\dot{V}O_2$ kinetics (and by inference $\dot{Q}musO_2$).

The mechanism behind the reduction in the ATP requirement for the same level of muscular work described by Rossiter *et al* (2003) is unclear. The authors suggest that a reduction in acid stress in the DCA trial due to a reduction in build up of fatigue metabolites Pi, H^+ or H_2PO_4 may affect a shift towards more efficient muscle fibres. This in turn would necessitate a lessened requirement for continued (and additional) fibre recruitment throughout the exercise period, leading to an improved $\Delta\dot{V}O_2 / \Delta WK$ with DCA infusion. No effect of DCA was observed on the fundamental delta $\dot{V}O_2$ or time constant for sub-threshold or supra-threshold exercise in chapters 3 and 4. Therefore, no effect of DCA on the oxygen deficit is supported by these results. As discussed previously, the discrepancy between these results may relate to the mode of exercise i.e. supine knee extensor versus upright cycling (refer to section 5.3).

6.5 The effect of DCA on the $\dot{V}O_2$ slow component

Casaburi *et al* (1987) and Poole *et al* (1990) showed that the reduction in the $\dot{V}O_2$ slow component with training was associated with the reduction in blood [lactate]. Therefore, it was hypothesised that the demonstration of a reduction in acid stress in the exercising muscle with DCA administration (Howlett *et al.*, 1999; Timmons *et al.*, 1996; Timmons *et al.*, 1998; Rossiter *et al.*, 2003) may lead to a decrease in the $\dot{V}O_2$ slow component. This was evident in the study of Rossiter *et al* (2003) who reported a ~28% reduction in the $\dot{V}O_2$ slow component, accompanied by a 32% decrease in end

exercise blood [La]. The proposed mechanism behind this reduction was thought to relate to a reduced requirement for recruitment of low oxidatively efficient muscle fibers (presumably type II), due to decrease in concentration of metabolites predisposing the exercising muscle to fatigue (Rossiter *et al.*, 2003). Direct measures of QO_2 (Poole *et al.*, 1991) & P-NMRS (Rossiter *et* al., 2001; Rossiter *et al.*, 2002) have demonstrated that ~85-90% of the $\dot{V}O_2$ slow component originates in the exercising muscle. The recruitment of fast twitch type II muscle fibers during high-intensity exercise has long been proposed to be a potential major factor in the emergence of the $\dot{V}O_2$ slow component (Whipp, 1994). However, when Rossiter *et al* (2003) expressed the $\dot{V}O_2$ slow component as a % of the fundamental amplitude the magnitude between CON and DCA not significantly differ. This is in agreement with the results from chapter 4 which clearly showed no difference in the $\dot{V}O_2$ responses during ~20min of supra-threshold exercise. These results and those from Rossiter *et al* (2003) and Jones *et al* (2000, 2004) may support a theory that the fundamental gain may play a role in modulating the magnitude of the $\dot{V}O_2$ slow component response. However, more research is needed in this area before any link can be realistically hypothesised.

6.6 Putative controllers of the oxygen uptake kinetic response

The question of what mechanism or, more likely, what mechanisms control the rate of oxidative phosphorylation remains. Kindig *et al* (2002) and Jones *et al* (2003) have demonstrated that a degree of control can be exerted through inhibition of nitric oxide synthase through L-NAME administration. Studies manipulating O_2 delivery to working muscles, commonly through the increase or decrease of FIO_2, have also shown this to be limiting when PO_2 falls below normoxic levels (Linnarson, 1974; Lawler,

1988; Roca, 1992). Similarly, the reported speeding of the supra-threshold $\dot{V}O_2$ kinetic response (although debated see Burnley *et al.*, 2000) after both hard (Gerbino *et al*, 1996) and moderate (Koppo and Bouckaert, 2000) prior exercise may also suggest that O_2 availability plays a role in determining the rate of the $\dot{V}O_2$ response at higher work rates (Grassi *et al.*, 2000). Furthermore, the findings of Howlett and Hogan (2003) demonstrating an increased rate of fall in PO_2 following DCA administration in isolated *Xenopus* frog muscle, do not discount a partial limitation to O_2 uptake due to a lag in PDH activation during the early stages of exercise.

The results of Kindig *et al* (2002) and Jones *et al* (2003) have demonstrated that controllers of the $\dot{V}O_2$ kinetic response can be borne out through the modelling of breath-by breath data. However, even the most significant of these effects - ~19% (Jones *et al.*, 2003)- does not identify this mechanism as a principal *loci* for control. Therefore, it is still likely that a greater degree of control may lie in feedback from products associated with the turnover of the high energy phosphate pool (Chance and Williams, 1955; Mahler M, 1985; Whipp and Mahler, 1990; Chance, 1979; Veach, 1979; Rossiter *et al.*, 2002; Rossiter *et al.*, 2003), and/or in the activation of enzymes in the TCA cycle or electron transport chain (Blomstrand *et al.*, 1997; Kindig *et al.*, 2002; Jones *et al.*, 2003; Brown, 1999).

6.7 The effect of Dichloroacetate on heart rate during exercise

Throughout this series of studies Dichloroacetate had a clear and consistent effect on heart rate. This effect was marked at rest and during sub maximal exercise. However at higher exercise intensities the effect was lost until perhaps at maximum exertion where all but one subject demonstrated a 1-3 beat increase in heart rate max.

An increase in heart rate in response to DCA administration was unexpected with many studies reporting no change in this measure (Stacpoole *et al.*, 1983; Wilson *et al.*, 1988; Wargovich *et al.*, 1988; Ludvik *et al.*, 1991; Ludvik *et al.*, 1993; Timmons *et al.*, 1998). Parolin *et al* (2000) however noted a 17 beat increase in heart rate at rest with DCA administration. Furthermore, Koppo *et al* (2004) reported a 7 bpm elevation in HR in the DCA condition compared with the control condition immediately prior to exercise. The magnitude of the heart rate increases in both studies is comparable with the results reported for the studies in this thesis.

Previous studies have indicated that DCA has a haemodynamic effect in humans at rest (Stacpoole *et al.*, 1983; Wargovich *et al.*, 1988; Ludvik *et al.*, 1991). Stacpoole *et al* (1983) reported an increase in systolic pressure and cardiac output in patients with hypotension and lactic acidosis. Wargovich *et al* (1988) demonstrated an increased left ventricular stroke volume, an improved myocardial efficiency and a decrease in systemic vascular resistance in patients with coronary artery disease. Ludvik *et al* (1991), in healthy individuals, found that cardiac index was increased by almost 20% at rest and that this was accompanied by a similar reduction in peripheral vascular resistance. Stacpoole (1989) has also reported evidence indicating DCA as a potent vasodilator.

Two mechanisms may be responsible for an increase in heart rate with DCA administration 1) a direct stimulatory effect of DCA upon the heart 2) peripheral vascular changes.

A direct stimulatory effect on the heart seems unlikely as an increase in heart rate would be expected to have been reported more frequently in previous studies and Stacpoole (1989) has reported evidence from isolated animal heart preparations that showed increases in cardiac function with no change in heart rate.

A reduction in stroke volume as a result of a drop in peripheral vascular resistance would also necessitate a rise in heart rate to maintain cardiac output. As the early cardiovascular response to exercise is mainly due to an increase in stroke volume, a response that is lost after about 40% of $\dot{V}O_2$ peak, this compensatory increase in heart rate would be expected to be more evident at lower exercise intensities. As described, the heart rate responses in these studies would fit this hypothesis.

The reported increases in stroke volume upon DCA administration are contradictory to this theory. However a possibility for the disparity between the results of this series of studies and those showing no augmented heart rate response is the body position associated with the measurements in these studies. Stacpoole (1983) and Wargovich *et al* (1988) used patient populations. While the position of these patients during measurement was not reported, considering their health it is likely that they were bed bound and therefore supine. Similarly, Timmons *et al* (1998) and Rossiter *et al* (2003) utilised a protocol requiring the subjects to assume a prone position. Ludvik *et al* (1991) did not report the subject position for measurement, however it was reported that

blood pressure measurements were performed in the supine position. With the body in this orientation stroke volume is already high at rest and this may be enough to negate the effect DCA may have on heart rate in the upright posture (these studies and Parolin *et al.*, 2000). In addition, the increases in stroke volume demonstrated in the study by Wargovich *et al* (1988) and inferred in the study of Ludvick *et al* (1991) may not be enough to compensate for the need to increase heart rate during rest and early stages of exercise in an upright orientation.

The reason for the heart rate increase in these studies remains unclear however it may have a functional significance by improving bulk oxygen delivery. While this is not thought to influence the oxygen uptake response in healthy populations under 'normal' conditions (Grassi, 2000), it may have implications in patient populations or hypoxia conditions where an O_2 limitation may be significant. Timmons *et al* (1996, 1997) present evidence which suggests that an improved oxygen delivery upon DCA administration is unlikely. Using a canine gracilis model they found no difference in blood flow and perfusion pressure after DCA administration. Similarly, Grassi *et al* (2002) using isolated canine gastrocnemius found no difference in the variables pertinent to O_2 transport between DCA and placebo trials. Furthermore, Bangsbo *et al* (2002) found no difference in thigh blood flow for the two conditions during knee extensor exercise. On the basis of the responses described in these studies, inferences made from isolated muscle preparations may not be applicable to the *in vivo* haemodynamic response. Furthermore the knee extensor exercise in the study of Bangsbo *et al* (2002) was performed on subjects in the supine position.

While as yet unsubstantiated, it has been suggested here that the haemodynamic effects of DCA may be highly dependant on posture. Therefore while the evidence from Timmons *et al* (1996, 1997), Grassi *et al* (2002) and Bangsbo *et al* (2002) support the notion that DCA has no effect on O_2 delivery further studies may be necessary before this can be substantiated. Therefore, at present, DCA should perhaps not be assumed to target an improvement in $\dot{V}O_2$ kinetics simply through an increase in substrate availability.

6.8 The Effect of Dichloroacetate on blood [lactate] during exercise

Dichloroacetate has previously been shown to be effective in reducing blood lactate at rest and during exercise (Merrill *et al.*, 1980; Parolin *et al.*, 2000; Stacpoole *et al.*, 1983; Durkot *et al.*, 1995; Schneider *et al.*, 1981; Carraro et *al.*, 1989; Hatta *et al.*, 1991; Roberts *et al.*, 1997; Rossiter *et al.*, 2003).

The results from these studies support the findings of Carraro *et al* (1989) and Hatta *et al* (1991) who demonstrated a reduced blood [lactate] during submaximal but not maximal exercise. This suggests that DCA is less effective at reducing blood lactate closer to peak exercise levels. The reduction in blood [lactate] through increased PDH activation is the result of 2 mechanisms 1) an increased rate of pyruvate catabolism increasing pyruvate flux into the mitochondria and subsequently reduction of blood [lactate] 2) a decrease in the rate of glycolysis. While a greater proportion of this lactate oxidation takes place in the skeletal muscle (Atomi *et al.*, 1987; Ivy *et al.*, 1980). DCA is effective in activating PDH in most mammalian tissues (Stacpoole, 1989). Therefore lactate oxidation in tissues such as heart and liver may also be increased.

Bangsbo *et al* (2002) reported PDH activation remained ~ 130% and 100% higher after 5 s and 15 s of severe (~110% $\dot{V}O_2$ peak) exercise but showed no difference 3 min into the exercise bout. Similarly, Savasi *et al* (2002) found PDH activation to be increased by DCA 30s into very heavy exercise (90% $\dot{V}O_{2\,max}$) but found no difference 90s into exercise. Indeed, Howlett *et al* (1998) demonstrated that PDH transformation into its active form is directly related to increasing power output and Constatin-Teodosiu *et al* (1991) observed complete PDH activation occurs at a workload of ~75% $\dot{V}O_{2\,max}$.

The results of these 3 studies support the original concept of Brooks (1985) who advocated that blood [lactate] reflected the balance between lactate production and removal. The reduction of blood [lactate] at rest, and during sub-maximal exercise, with DCA administration demonstrate a capacity for increased lactate removal through activation of PDH. How much the decreased blood [lactate] reflects an increased oxidative capacity of exercising or non exercising contractile muscle units, or represents a greater contribution from non muscular lactate oxidation cannot be determined. Timmons *et al* (1998) noted that during submaximal knee extensor exercise blood lactate was reduced after DCA administration, however, muscle [lactate] in this study remained unchanged. Similarly Bangsbo *et al* (2002) reported decreases in venous [lactate] with no change in muscle lactate production. Therefore it is likely that PDH activation in inactive and partly active muscle fibres, in conjunction with other body tissues, leads to a greater conversion of lactate to pyruvate and not to a decreased lactate production.

The results from the incremental study agree with this, with the point of lactate increase (and subsequently θ_{LT}) being similar between DCA and placebo conditions, despite a ~50% reduction in resting blood [lactate] in the DCA study. This was coupled with no

observable difference between the rise in muscle deoxyhaemoglobin between the two trials. This is further supported by the submaximal blood [lactate] values being lower in both the constant work rate studies and during the submaximal portion of the ramp, with no observable difference at maximal work intensities. This is consistent with the data of Carraro *et al* (1989) who found no difference in maximal blood [lactate] and the data from Rossiter *et al* (2003) and Hatta *et al* (1991) who showed blood [lactate] to be reduced throughout submaximal exercise.

Therefore there is evidence for a lactate clearance reserve that can be reduced through activation of PDH during submaximal exercise intensities. This decrease persists until perhaps such levels that clearance has reached a maximal level and PDH activation no longer effects lactate removal. These results partly support the 'lactate shuttle' hypothesis of Brooks (1986) advocating that lactate removal plays an important role in blood [lactate] both at rest and during exercise. However the results also support the theories that suggest while lactate production / removal does play a role in determining the absolute blood [lactate] it is not a primary cause of increased lactate production (Wasserman and McIlroy, 1964; Katz and Sahlin, 1988; Bonen *et al*, 1997; Gladden, 2004).

6.9 Conclusions

This series of studies have demonstrated dichloroacetate to have no significant effect on submaximal oxygen uptake kinetics during dynamic exercise in humans. This was evidenced as no change in the amplitude or time course of the fundamental phase II response during exercise above or below the lactate threshold (θ_{LT}), and no change in the slow component response above the lactate threshold. DCA administration did promote a small but significant increase in $\dot{V}O_{2max}$, possibly due to an augmented cardiac output. Control of oxygen uptake kinetics at the level of pyruvate dehydrogenase is therefore not supported by these studies. However, a degree of influence from PDH activation during the initial phase (10-20s) could not be ruled out (Howlett and Hogan, 2003). The differences between these studies and those that have report an improvement in muscle efficiency during whole body exercise (Grassi., 2002; Rossiter *et al.*,2003; Jones *et al.*, 2003) may be explained by the mode and intensity of the exercise employed. This has implication for further studies looking at kinetic control and the conclusions that can be made from this area of study.

Blood [Lactate] was reduced at submaximal exercise intensities. However, no significant difference was evident during maximal exercise. This suggests a greater clearance of blood lactate due to up regulation of PDH by dichloroacetate and not a reduction in lactate production. This would appear to highlight a possible 'lactate clearance reserve' during exercise.

Exercise time to exhaustion was not significantly different between the two trials during supra threshold exercise, indicating that DCA is not associated with a performance effect for this subject group and the exercise mode employed. However the small

improvement in $\dot{V}O_{2max}$ suggests that further investigation into the ergogenic effects of DCA may be warranted. The reported improvements in muscular performance reported during conditions of hypoxia or ischaemia, and the possibility of improvements in bulk O_2 delivery indicated by this study, suggest that the while DCA appears to have little effect under 'normal' energetic conditions, a significant improvement in muscular performance may be realised when 'normal' function is constrained. This may improve exercise performance in patient groups with limited aerobic capability or athletic/patient populations working under hypoxic or anaerobic conditions.

DCA was shown to have haemodynamic properties evident by an observed increase in heart rate at rest and during the early stages of exercise above and below the lactate threshold. This may indicate changes in cardiac output during this period. These effects of DCA have not previously been described and these results suggest that further investigation in this area is needed.

The notion that a limitation to the rate of oxidative phosphorylation at the onset of exercise exists at the level of pyruvate dehydrogenase is not supported by the results from these studies. Therefore the principle rate limiting step for this mechanism must lie at a level beyond this point.

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UNIVERSITY OF GLASGOW

ETHICS COMMITTEE FOR NON CLINICAL
RESEARCH INVOLVING HUMAN SUBJECTS

RESEARCH SUBMISSION

Name of person(s) submitting research proposal **Dr Yannis Pitsiladis, Professor Susan Ward**

Position held **Lecturer in IBLS, Director of CESAME and Professor of Exercise Science and Medicine**

Department/Group/Institute/Centre - **Centre for Exercise Science and Medicine (CESAME), IBLS**

Name of Principal Researcher (if different from above) _____

Position held _____

Date of submission **June, 1999**

Project Title

Effects of dichloroacetate on metabolic parameters during incremental and constant-load exercise in healthy human subjects.

1. Describe the basic purposes of the research proposed.

Background of investigation

Dichloroacetate (DCA) is a potent stimulator of pyruvate dehydrogenase, a mitochondrial enzyme which catalyses metabolism of pyruvate and lactate and inhibits glycolysis (for review, see ref 1). Adminstration of DCA has been shown to significantly reduce blood lactate levels in healthy volunteers (2) and in patients with lactic acidosis (3, 4). Additionally, DCA stimulates myocardial contractility and decreases peripheral vascular resistance (4) thereby increasing muscular oxygen delivery. Systemic limitations to intense exercise may result from either insufficient oxygen availability or from the metabolic consequences of those reactions which provide the alternative energy for the oxygen which is required but not utilised. Given these metabolic and haemodynamic effects, DCA would be expected to influence exercise tolerance by increasing muscle oxygen availability and reducing muscle lactate, and therefore, hydrogen ion production. This hypothesis will be tested in normal healthy subjects by examining the effects of DCA on the lactate threshold, maximal oxygen uptake and the metabolic and acid-base response profiles to constant-load exercise below and above the "lactate threshold" (LT - the highest work rate at which there is no sustained increase in blood lactate) (6).

2. Outline the design and methodology of the project. Please include in this section details of the proposed sample size.

Methods/Design of Investigation

We propose to study eight normal healthy male subjects aged between 18 and 35 years (this sample size is in line with the statistical procedures to be used). All subjects will be in good health at the time of testing and take part in at least two aerobic exercise sessions per week. Subject elligibility will be assessed by subjects completing a medical and a physical activity questionnaire. Subjects will also be required to read and sign the enclosed information sheet.

Testing will take place in the Exercise Physiology laboratory in the West Medical Building. All subjects will perform three incremental and three constant-load cycle ergometer tests, which will be carried out in the late afternoon on six separate occasions. The first maximal incremental and constant-load cycle ergometer tests are aimed at familiarising subjects with the exercise protocol and experimental procedures. One hour prior to the second and third maximal incremental and constant-load exercise tests (see protocols below), subjects will consume either a 50mg·kg^{-1} body weight acute dose of DCA or an equivalent amount of placebo. Test solutions will be administered to the subjects in the laboratory in a double blind fashion. The DCA oral formulation to be used will be prepared by pharmacy at the Western Infirmary. Subjects will consume their normal diet throughout the study period and will be required to refrain from all strenuous exercise in the 24 h period prior to each test.

Protocols

Maximal incremental exercise test: On three occasions, subjects will perform a maximal incremental exercise test, as follows. A direct measurement of maximal oxygen uptake ($\dot{V}_{O2}$ max) and the lactate threshold will be determined on a computer controlled cycle ergometer (Lode). This test will involve a step-wise increase in workrate (e.g. 15-20 watts·min^{-1}) until volitional exhaustion. The results from this test will allow the lactate threshold to be estimated using gas exchange criteria. Subjects will be given a familiarisation trial and a warm-up before the test and a warm-down after the test.

Submaximal tests - constant load: On three separate occasion, subjects will perform two consecutive bouts of constant-load exercise at a moderate exercise intensity (below the lactate threshold) for 10 minutes, followed by one bout of constant-load exercise at a high exercise intensity (above the lactate threshold) designed to induce fatigue within about 10 minutes. Ten minute periods of unloading cycling will be carried out between the three exercise bouts. The results from this test will allow the gas exchange kinetics, metabolic and perceptual responses of the two exercise domains to be determined (see measurements below). Subjects will be given a familiarisation trial and a warm-up before the test and a warm-down after the test.

Measurements

Cardiovascular Measurements: Heart rate will be monitored by electrocardiography.

Respired Air Measuremnts: Respired air will be measured continuously for volume (turbine) and oxygen (O_2) and carbon dioxide (CO_2) and nitrogen (N_2) concentration using a mass spectrometer for on-line breath-by breath determination of O_2 uptake ($\dot{V}_{O2}$) CO_2 output ($\dot{V}CO_2$) and ventialtion ($\dot{V}_E$) and alveolar PO_2 and CO_2 partial pressure (PO_2, CO_2) (QP9000, Morgan Medical).

Breathlessness and Rating of Perceived Exertion: Breathlessness and Rating of Perceived Exertion will be monitored at regular intervals throughout the tests, using either a standard Borg or visual analogue scale.

Arterial oxygenation measurements: Arterial blood oxygen saturation will be monitiored continuously, noninvasively from a finger or an ear lobe by pulse oximetry.

Muscle Oxygenation: Intravascular oxygen saturation will be monitored continuously and noninvasively from the surface of the right or left quadriceps muscle using near infra-red spectroscopy (Hamamatsu).

Blood Analysis: Arterialised-venous blood samples will be drawn at rest and at 5 min intervals during exercise from a 20 G indwelling catheter placed by percutaneous puncture into a vein on the dorsum of the heated hand (this yields blood with a composition indistinguishable from arterial, but without the associated degree of invasion and therefore subject discomfort) (7, 8). Blood samples (5 ml or 1 teaspoon each) will be analysed for blood gas and acid-base variables, blood lactate and pyruvate (9), catecholamines (10), and prolactin (Technicon Immuno 1 System, Bayer Diagnostics, Newbury, UK). The

3. Describe the research procedures as they affect the research subject and any other parties involved.

All tests will take place in the Exercise Physiology Laboratory, West Medical Building. Dr Yannis Pitsiladis and/or Professor Sue Ward will be present at all tests. Both are certified phlebotomists and trained in CPR. Before all exercise tests, a medically qualified person will be informed of the time of the test and asked if he/she is available. Exercise tests specific to this study will only be conducted if a medically qualified person can be contacted and is able to be present within a short time period. A defibrillator and emergency drugs are on site in the laboratory.

Some subjects may experience mild discomfort during the placement of and/or sampling of blood from a catheter placed in a vein on the dorsum of the heated hand. In our experience, this is minimal because: the catheter size is small (20 G); it is only placed when the hand has been heated to 44° C for at least 10 min, allowing a substantial local vasodilatation of the superficial blood vessels, which facilitates their cannulation; the catheter is indwelling, allowing for multiple sampling; while it is safely secured in place with adhesive tape, there is sufficient 'play' to allow sampling without 'pulling' on the vessel (i.e. the catheter can slide easily within the vessel); upon withdrawal of the catheter at the end of the experiment, firm pressure is maintained over the site for at least 15 min to prevent any leakage from the vessel into the surrounding interstitium which could lead to local oedema and bruising. Importantly, if a vessel cannot be readily cannulated or if the subject is nonetheless not comfortable with proceeding, the experiment is halted. No more than 100 ml or 20 teaspoons of blood will be sampled for each test.

Some subjects may experience a mild tranquilizing or sedative effect following the administration of DCA. In most cases, the effect is short lived and is too slight to be measured by objective tests that are sufficiently sensitive to detect early stages in cognitive dysfunction induced by sedative hypnotics or tricyclic antidepressants (1). In occasional subjects who metabolise the drug slowly, drowsiness may persist for several hours (1). Subjects will not be permitted to leave the laboratory until fully recovered.

Potential participants will be identified either by personal contact or by advertisement. They will be asked to meet with the investigators to discuss the project and whether they would be suitable as a subject. All subjects will be healthy individuals without a history of any significant medical problem(s). All subjects will be moderately-trained and therefore accustomed to strenuous exercise. The good health of each subject will be established prior to the study by taking the subject's medical history, which is supported by a written assurance from the subject. Subjects with a history of cardiorespiratory or neurological disease will be excluded from participation, as will those having an acute upper respiratory tract infection. Subjects who take drugs (recreational or performance enhancing drugs) or who have consumed alcohol within 48 h of an experiment will be excluded.

Close supervision of the subject is ensured at all times by the supervising investigator. The well-being of the subject is established at frequent intervals throughout all tests by asking the subject "Is everything alright?". Subjects are instructed, prior to the test, to respond to this question with a thumbs-up sign if everything is fine, and a thumbs-down sign if there is problem. If a problem is indicated, the investigator will ask further questions to establish whether there is a technical problem that could lead to potential hazard or whether the subject is feeling unwell. In either case, the test is immediately halted.

All subjects are routinely instructed to cease exercising if they experience any discomfort or have any concern for their well-being.

The risks associated with performing maximal exercise are minimal as long as the subject is appropriately instructed and familiarised with the device prior to participation and also is appropriately supervised during the experiment. All exercise bouts are both preceded by a 5 min "warm-up" and by a 5 min "warm-down". The latter is of particular importance during high-intensity exercise, when the local accumulation of exercise metabolites can cause an "expansion" (or vasodilatation) of the blood vessels in the lower limbs, which can impair the adequate return of blood to the heart - predisposing to fainting on dismounting from the ergometer. This risk is minimised by having the subject exercise at a mild level during recovery to "wash away" these metabolites and therefore to restore the capacity of the involved blood vessels to their resting levels.

Some subjects experience difficulty swallowing while breathing through a mouthpiece and wearing a noseclip, due to some transient build-up of pressure in the ears.

Some subjects experience increased salivation when breathing through a mouthpiece.

Some subjects experience mild discomfort from prolonged sitting on the seat of the cycle ergometer.

4. What in your opinion are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

Administration of DCA in human subjects has been limited to short term studies in healthy volunteers, adults with type II diabetes, and children and adults with various forms of lactic acidosis (for review, see ref 1). Aside from typically mild drowsiness in about one half the adult recipients, no other drug related adverse effects are reported. There have been claims that unpurified preparations of DCA may be mutagenic (11-14). However, studies using DCA of established purity failed to show any mutagenicity (15). There was one case of peripheral neuropathy in a young adult man with homozygous familial hypercholesterolemia who received single daily doses of 50 $mg \cdot kg^{-1}$ body weight DCA for up to four months (16). Toxicity was manifested by loss of deep tendon reflexes, mild weakness of facial and interosseous muscles, and diminished tibial nerve conduction velocity. This neuropathy was resolved several weeks after cessation of therapy, suggesting that functional rather than morphologic changes were responsible for the toxicity. Furtheremore, two infants with severe neuropathy and congenital forms of lactic acidosis were treated for approximately 1 year with similar doses of DCA with no adverse effects (17, 18). In summary, acute doses of 50 $mg \cdot kg^{-1}$ body weight DCA administered orally or intravenously is safe and represents a valuable tool for exploring the metabolic pathways both in health and disease.

Exercise has negligible risk in healthy adults, although maximal exercise has a small risk of inducing myocardial ischaemia.

The insertion of a catheter into a vein may rarely cause irritation at the site of insertion, venospasm (or constriction of the cannulated vein which may lead to interference with blood flow through it) and phlebitis. These risks are minimized in this investigation by the short duration of the test and by the procedures described above.

Blood will be handled, stored and disposed of according to standard health and safety procedures.

5. Outline the reasons which lead you to be satisfied that the possible benefits to be gained from the project justify any risks or discomforts involved.

The research undertaken will allow gas exchange kinetics, metabolic and perceptual responses to maximal incremental and constant-load exercise following DCA administration to be determined and compard to normal responses. It is envisaged that this research will benefit the identification of the physiological mechanisms which limit exercise tolerance (i.e. the ability of individuals to perform exercise) in health and disease. The minimal risk and discomfort associated with the above procedures are considered to be worthwhile to gain the information required.

6. Who are the investigators (including assistants) who will conduct the research and what are their qualifications and experience?

Professor Susan Ward BSc BA D Phil, Dr Yannis Pitsiladis PhD MMedSci BA, Mr John Wilson and Mrs Heather Collin (Senior Technicians). The principal investigators have wide ranging experience of exercise testing over periods of up to 25 years without incident.

7. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

A clinician will be alerted before all experiments and can be in attendance within a short space of time. The Exercise Physiology laboratory has a set of instructions fixed to the wall next to the telephone, so that the caller can identify him/herself and the location of the laboratory.

In the event of an untoward incident that is not an emergency, the supervising Principal Investigator will administer appropriate first aid, if necessary. The subject will not be permitted to leave the laboratory until he has fully recovered. The subject will be encouraged to contact his local GP. The subject will be told that one of the Principal Investigators will conduct a follow-up by telephone at the end of the same day. The subject will also be provided with 24-hour contact numbers for both Principal Investigators.

8. In cases where subjects are identified from information held by another party (for example, a doctor or hospital) describe the arrangements whereby you gain access to this information.

N/A

9. Specify whether subjects will include students or others in a dependent relationship.

Some students may be recruited but will be under no pressure from staff to participate in the study.

10. Specify whether the research will include children or those with mental illness, disability or handicap. If so, please explain the necessity of using these subjects.

N/A

11. Will payment be made to any research subject? If so, please state the level of payment to be made, and the source of the funds to be used to make the payment.

NO

12. Describe the procedures to be used in obtaining a valid consent from the subject. Please supply a copy of the information sheet provided to the individual subject.

Each subject will recieve an information sheet outlining the tests to be performed (see enclosed copy). A verbal explanation will also be given and any queries answered.

13. Comment on any cultural, social or gender-based characteristics of the subject which have affected the design of the project or which may affect its conduct.

None

14. Give details of the measures which will be adopted to maintain the confidentiality of the research subject.

The information obtained will be anonymised and individual information will not be passed on to anyone outside the study group. The results of the tests will not be used for selection purposes.

15. Will the information gained be anonymized? If not, please justify.

Yes

16. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

No

17. Date on which the project will begin (1 August, 1999) and end (20th December, 1999)

18. Please state location(s) where the project will be carried out.

Exercise Physiology laboratory, West Medical Building

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INFORMATION SHEET

TITLE OF INVESTIGATION: Effects of dichloroacetate on metabolic parameters during incremental and constant-load exercise in healthy human subjects.

We invite you to participate in an investigation which we believe to be of potential importance. In order to help you to understand what the investigation is about, we are providing you with the following information. Be sure you understand it before you formally agree to participate. Ask any questions you have about the information which follows. We will do our best to explain and to provide any further information you require. You have been selected as a possible participant in this investigation because you are in good health.

The mechanisms that determine the ability to sustain moderate to severe exercise are poorly understood. Such information, however, is crucial if we are to improve exercise tolerance (i.e. the ability of individuals to perform exercise) in both health (e.g. the performance of the elite athlete) and disease (e.g. patients with compromised energy metabolism). This study aims to compare a range of responses to different intensities and duration of exercise following the administration of a drug known to influence the chemical reactions involved in energy production.

Testing will take place in the Exercise Physiology laboratory, West Medical Building at Glasgow University. You will be asked to visit the laboratory on six occasions and to take part in the following tests:

Progressive Exercise Test: You will be asked to perform a maximal progressive exercise test on a stationary computer controlled cycle so that we can assess your level of fitness (i.e. maximal oxygen uptake and the lactate threshold). During this test, the pedaling load increases over the course of 15-25 minutes until you have to stop cycling either because of fatigue or breathlessness. You will repeat this test on two further occasions. One hour prior to commencement of these two subsequent tests you will be required to consume 100 ml of a drink containing either 50mg/kg of your body weight of a drug called Dichloroacetate (DCA) or an equivalent amount of an inert placebo.

Submaximal test - constant load test: On three separate days, you will cycle at moderate and high exercise intensities (below and above the lactate threshold) for periods ranging between 5 - 10 minutes, with 10 minutes of unloaded cycling between the exercise bouts. The total test duration will be no more than one hour. The first constant-load test aims to familiarise you with the exercise protocol and experimental procedures. One hour prior to the next two constant-load exercise tests you will again be required to consume 100 ml of the drink containing the drug or an equivalent amount of the placebo.

DCA is a drug which effects the energy production pathways and reduces the amount of lactic acid produced (lactic acid is a breakdown product of anaerobic metabolism). This drug is used in the treatment of lactic acidosis. DCA may cause you to become a little drowsy, however these effects are normally mild and do not persist for very long.

During each exercise test and at regular intervals, we would like to take a small amount of blood from an intravenous line in the back of your hand. Intravenous lines may cause some bruising and subsequent soreness over the site of puncture and, rarely, a small wound which takes a few days to heal.

Arterial blood oxygen saturation will be monitored continuously noninvasively from a finger or an ear lobe by pulse oximetry. Intravascular oxygen saturation will be monitored continuously and noninvasively from the surface of the right or left quadriceps muscle using near infra-red spectroscopy (Hamamatsu).

Breathlessness and Rating of Perceived Exertion will be monitored at intervals throughout tests, using either a standard Borg (i.e. numbers with word anchors to help you rate a variable) or a visual analogue scale (VAS) (the VAS scale consists of a horizontal line. The word "none" is placed at one end of the scale and the word "very severe" at the other). You will be asked provide a response which relates to your level of Breathlessness and Rating of Perceived Exertion using these scales.

During the exercise tests, you may breathe through a rubber mouthpiece which is similar to that used for snorkeling, and wear a noseclip. You may experience difficulty swallowing while breathing through a mouthpiece and wearing a noseclip, due to some pressure in the ears. Some subjects experience increased salivation when breathing through a mouthpiece. Some subjects experience mild discomfort from prolonged sitting on the seat of the cycle ergometer.

Exercise has a negligible risk in healthy adults, although maximal exercise has a small risk of inducing myocardial ischaemia. The primary symptom of myocardial ischaemia is chest pain on exertion. If you experience any unusual sensations in your chest during the experiment, you should cease exercising immediately. Your heart rate will be monitored via adhesive surface electrodes for the monitoring of the heart's electrical activity (the "electrocardiogram").

Before you become a subject, you will complete a medical questionnaire. People who have asthma, heart related and/or circulatory problems, hypertension or any other contraindicated condition will not be allowed to take part in the study.

All information obtained both from the preliminary medical questionnaire and from the study itself will be treated confidentially. It is our intention to publish the results of this study, but not in a way which will not enable individuals or their performance to be identified.

You are free to leave the study at any time. The outcome of the study may not benefit you directly. Some parts of the study constitute a possible transient risk to your health. There is a small cardiac risk to your health. You may feel uncomfortable during certain stages of the tests.

If you are worried about any unwanted side effects from any of the above procedures, you should contact:

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Consent Form

I .....

give my consent to the research procedures which are outlined above, the aim, procedures and possible consequences of which have been outlined to me

Signature .....

Date .....

Appendix 2

CENTRE FOR EXERCISE SCIENCE AND MEDICINE

MEDICAL HISTORY

(CONFIDENTIAL)

Please read.

It is important to take a record of your medical history. You may have, or may have once had a condition that would make this type of testing unsuitable for you. For this reason we ask you to be as truthful and detailed as possible. At no point will this information be made available to any one other than the principal investigators for this study. If you have any doubts or questions, please ask.

SUBJECT DETAILS:

NAME:

AGE: D.O.B:

SEX (M/F):

GP NAME & ADDRESS:

SMOKING:

Never Smoked .....
Not for >6 months .....
Smoke <10 per day .....
Smoke >10 per day .....

ILLNESSES:

ALLERGIES:

HOSPITALISATIONS:

MUSCULO-SKELETAL DISORDER:
(Arthritis, Joint Pain, Fractures, Sports injury, Others)

CARDIOVASCULAR DISORDER: (Fever, Heart Murmurs, Chest Pain, Palpitations, High Blood Pressure, Others)

RESPIRATORY DISORDER: (Asthma, Shortness of Breath (SOB), Cough, Upper Respiratory Tract Infection (URTI), Others)

GASTROINTESTINAL DISORDER: (Jaundice, Bleeding, Others)

DIABETES:

CNS DISORDER: (Fits, Blackouts, Tremor, Paralysis, Epilepsy, Other)

PSYCHIATRIC TREATMENT:

FAMILY HISTORY: (Sudden death in a first degree relative under the age of 35 years)

ARE YOU CURRENTLY TAKING ANY MEDICATION? No / Yes*

(*Please specify)_____

ARE YOU CURRENTLY TAKING ANY SUBSTANCES TO HELP IMPROVE YOUR TRAINING OR CONTROL YOUR WEIGHT i.e. CREATINE. PROTEIN SUPPLEMENT? No / Yes*

(*Please specify) _____

ARE YOU CURRENTLY TAKING ANY OTHER SUPPLEMENTS i.e. FOOD SUPPLEMENTS, VITAMINS? No / Yes*

(*Please specify) _____

CAN YOU THINK OF ANY OTHER REASON WHY YOU SHOULD NOT TAKE PART IN ANY OF OUR TESTS?

_____

SYMPTOMS:

Do you experience any of the following, particularly on exercise?

Breathlessness	No / Yes
Chest Pain	No / Yes
Dizzy Fits/Fainting	No / Yes
Palpitations	No / Yes

Please note that if you feel unwell on the day of the proposed test, or have been feeling poorly over the preceding day or two, please inform the investigators and DO NOT TAKE PART in the exercise test.

DECLARATION:

I have completed this questionnaire fully and truthfully. I have not kept any information from the investigators that may put myself at risk during high-intensity exercise, or affect the results that they obtain. I understand that I may withdraw from any one test or the study as a whole if I feel unwell, or feel uncomfortable with any part of the testing procedure.

(Signature).....

(Date) .....

PHYSICAL EXAM:

WEIGHT: _____ HEIGHT: _____

PULSE (Resting): _____ BP (Resting): _____

Screened by: .....

(Signature) ..... (Date) .....